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THE UNIVERSITY OF ALBERTA

THRESHOLD DETECTION VALUES OF POTENTIAL FISH TAINTING SUBSTANCES FROM OIL SANDS WASTEWATERS

BY

CYNTHIA GAY JARDINE

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND
RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE

IN

ENVIRONMENTAL SCIENCE

DEPARTMENT OF CIVIL ENGINEERING

EDMONTON, ALBERTA

SPRING 1988

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Threshold Detection Values of Potential Fish Tainting Substances from Oil Sands Wastewaters** submitted by **Cynthia Gay Jardine** in partial fulfillment of the requirements for the degree of **Master of Science** in **Environmental Science**

.....
Supervisor

.....

.....

.....

Date: Mar 31/88

To Errol

for his patience, support and motivation,

To my parents, Archie and Marie

for always encouraging me to be the best I can be,

and

To my brother and sister, Scott and Janet

for their love and support

ABSTRACT

Chemical compounds associated with oil sands extraction and upgrading operations have been implicated in fish tainting problems in the Athabasca River, north of Fort McMurray, Alberta. This study determined detection threshold levels for selected compounds judged to be candidate tainting compounds in oil sands wastewaters. These compounds were spiked in walleye (*Stizostedium vitreum*) flesh and subjected to odour detection by a screened and trained sensory panel of eleven members.

A preliminary examination of sensory evaluation methods determined the Consistent Series Test to be a more sensitive test for threshold detection than the Modified Triangle Test.

Of the twelve compounds tested, four were not pursued for determination of threshold detection values because of poor detectability by the panelists in the preliminary tests. The taint detection thresholds of the other eight compounds ranged from 0.09 mg/kg for benzothiophene to 12.2 mg/kg for 2,6-dimethylnaphthalene. Repetition of the test for benzothiophene under different conditions produced similar thresholds (0.09 and 0.12 mg/kg), suggesting that the determined threshold values are independent of both the range of concentrations presented and nature of the carrier solvent used in spiking the flesh with the compounds.

Analysis of the spiked fish tissue showed that poor analytical recoveries of a compound can be directly related to increases in volatility, and that the analytical values may often underestimate the concentration of a specific compound in fish tissue. Good analytical recovery for relatively non-volatile compounds, such as

dibenzothiophene, confirmed the uniformity of the spiking technique used in sample preparation.

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Preliminary Panel: Bob Andrews, Eddie Doyle/Rick Nelson, Janet Kinsella, Cheryl Podemski and Errol Billing

Final Panel: Angelo Fernando, Gladys Ylimaki, Lynn McMullen, Terry Smith, Wayne Roberts, Mirella Cerrone, Suzanne Troxler, Charlotte Martynuik, Steve Hrudey and Errol Billing

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1. INTRODUCTION

1.1 BACKGROUND

Alberta, Canada has an estimated 210 x 10^9 m^3 of hydrocarbon reserves, of which about 200 x 10^9 m^3 (or 95%) is found as bitumen in four large oil sands deposits. The best known oil sands deposit is the Athabasca deposit. About 10% of the 112 x 10^9 m^3 in this deposit is covered by less than 60 m of overburden and is presently being surface mined. Two commercial-scale plants near Fort McMurray have been producing synthetic crude oil from the bitumen for 10 and 21 years, respectively. Chemical compounds associated with oil sands extraction and upgrading have been implicated in fish tainting problems in the Athabasca River, north of Fort McMurray.

The lack of adequate information on fish tainting concerns associated with oil sands wastewaters became apparent following a closure of the commercial fishery in 1982 because of petroleum-like off-flavours. This tainting incident was coincident with upset conditions at the Suncor oil sands plant during the winter of 1981-82 which resulted in a substantial discharge of hydrocarbons to the Athabasca River under ice conditions. The resulting investigation showed that little is known about the fish tainting potential of compounds present in the process and discharge waters.

As the older of the two plants, Suncor's present operating licence allows for discharge of upgrading plant wastewaters into the Athabasca River. The second oil sands operation, Syncrude Canada Ltd., has been operating under a requirement for zero discharge or total containment of wastewaters since start up in 1978. Further motivation for examining fish tainting concerns arose from the recent request of

Syncrude to Alberta Environment to establish standards/guidelines for treated wastewater discharge from the plant to the Athabasca River in anticipation of the eventual need to decommission the site and tailings pond area.

In response to the demand for additional quantitative information on the fish tainting concerns related to oils sands wastewaters, Alberta Environment has initiated a research program on the aquatic fate of hydrocarbon compounds in the Athabasca River. This research addresses one component of this program, specifically the nature and quantity of specific chemical compounds present in oil sands wastewaters that may result in fish tainting. Walleye (*Stizostedium vitreum*) tissue was spiked with a range of concentrations of each compound, and the threshold detection levels determined using a screened and trained sensory panel.

1.2 RESEARCH OBJECTIVES

The primary objective of this research was to determine the detection threshold values in fish tissue of specific chemical compounds which are present in oil sands wastewaters and are also suspected of being capable of tainting fish.

Secondary objectives were:

1. To determine the most appropriate sensory method for evaluating detection thresholds of hydrocarbons in fish tissue.
2. To develop suitable procedures for spiking and presenting the fish samples for sensory evaluation.
3. To assess the performance of a screened, trained sensory panel in threshold evaluations.

4. To consider actual detection threshold values with theoretical bioconcentration factors for specific hydrocarbon compounds.
5. To develop suitable analytical methods for determining the concentrations of specific hydrocarbon compounds in fish tissue.
6. To compare spiked and analytical concentrations of hydrocarbon compounds in fish tissue, and relate the derived threshold values to actual tainting occurrences.

1.3 STUDY AREA

The area of concern is the Athabasca River, north of Fort McMurray to the Peace-Athabasca Delta (Figure 1). This section of the river is the receiving water body for the discharge of the treated upgrading plant wastewaters from the Suncor plant and would be the receiving body of any future discharge of treated wastewaters from the Syncrude plant.

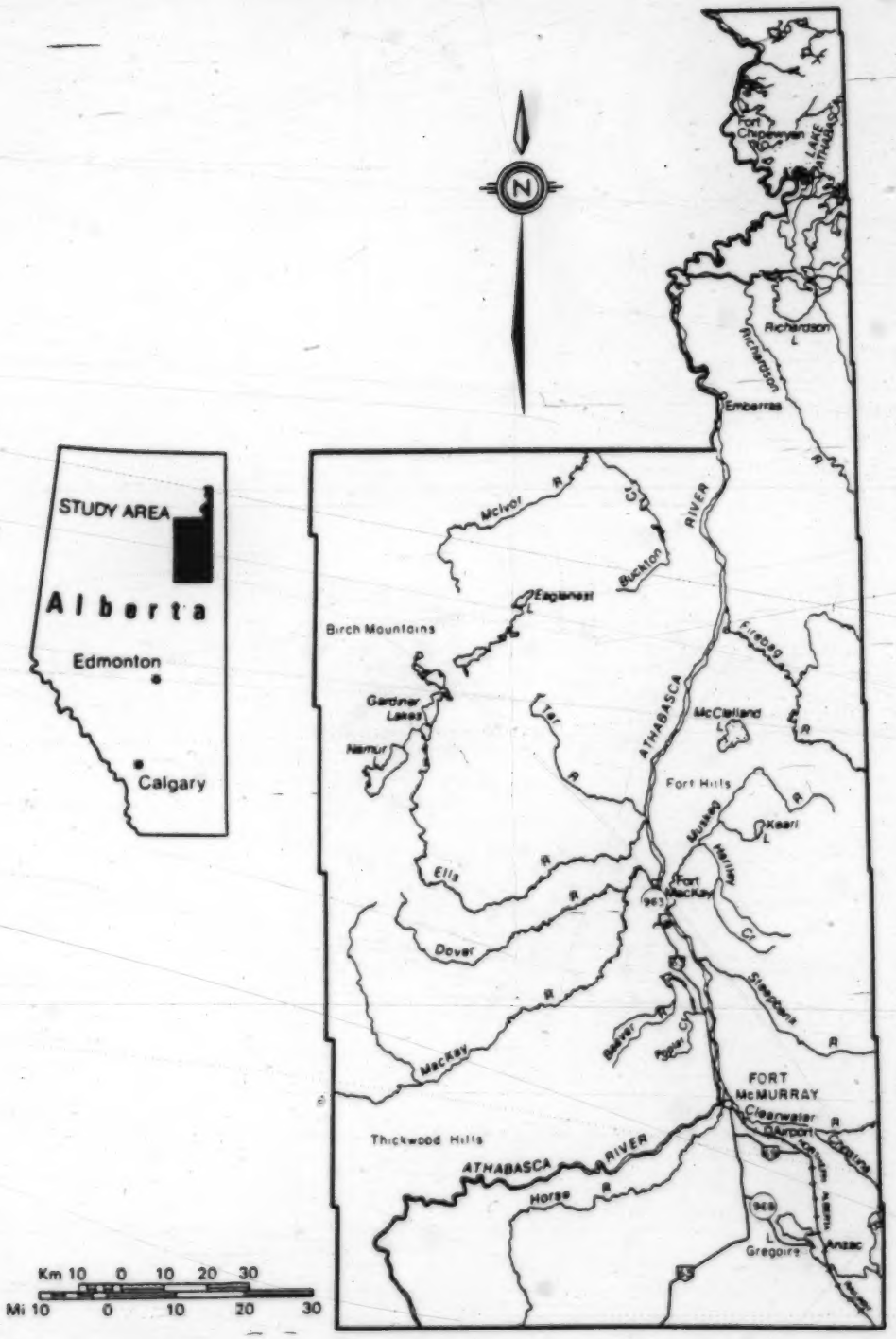


Figure 1. The study area (modified from Research Management Division, Alberta Environment).

2. LITERATURE REVIEW

2.1 PREVIOUS FISH TAINTING INCIDENTS

Tainting is defined as a change in the characteristic smell or flavour of fishes, and is of concern because it may decrease desirability and marketability of the commercial, domestic or recreational fisheries (Trudel 1986). Tainting is considered to exist when the concentration of any compound in fish tissue meets or exceeds the detection taste and/or odour threshold for that compound. The determination of detection threshold values of suspected tainting compounds is thus important for regulatory control and monitoring of potential sources of tainting compounds.

The problem of tainting of fish and other aquatic organisms from oil and petroleum compounds has been internationally recognized for many years. In 1982, the IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) (1982) established that: (a) crustaceans, fish and molluscs exposed to oily conditions can acquire an oily taste; (b) the taste is intimately associated with the presence of volatile compounds derived from oils or dispersants; and (c) the range and quantity of odourous compounds vary with the nature of oil.

The presence of an "oily" flavour has been correlated with the presence of petroleum hydrocarbons in fish flesh in areas around the world where there have been chronic discharges from petroleum refineries, ships or shore-based industrial facilities, or where spills of crude oil or refined petroleum products have occurred. Tainting has been associated with diesel fuel, crude oil, Bunker C, gasoline and refinery effluents (Tidmarsh and Ackman 1986)

In 1950, the occurrence of objectionable levels of an "oily" taste-flavour in the rainbow trout in the Bow River downstream from Calgary, Alberta was linked to petroleum refinery wastewater discharges. Preliminary studies done in 1958 confirmed that exposing rainbow trout to dilutions of the oil refinery effluent similar to those found in the Bow River caused tainting of the fish flesh (Anonymous 1958). In subsequent investigations, Krishnaswami and Kupchanko (1969) concluded that rainbow trout will acquire an oily taste-flavour within 24 hours if maintained continuously in a water in which the petroleum refinery wastewater is diluted to a final threshold odour number (TON) of greater than or equal to 0.25 (calculated value). This demonstrated that fish can acquire a taint from wastewater diluted below levels which are odourous.

In the spring of 1972, fish caught through the ice of the Athabasca River, downstream of Jasper, Alberta were judged unfit to eat by the local fishermen. The source of the taint was suspected to be a diesel fuel discharge pipe below the Canadian National Railway yards in Jasper. Chromatograms of the steam distillates from the tainted fish exhibited the same chemical components (primarily n-alkanes) present in the diesel oil (Ackman and Noble 1973).

A kerosene-like taint in sea mullet from Australian waters near Brisbane was initially reported by Grant (1969, cited in Connell 1974). In further investigations of this problem by Vale et al. (1970), Shipton et al. (1970) and Connell (1971, 1974), the volatile flavouring substances in the tainted mullet were shown by gas chromatography and mass spectrometry to be very similar to commercial kerosene. The source of the tainting was originally believed to be from the effluents of two refineries on the banks of the Brisbane River. However, Connell (1974) later isolated kerosene-like hydrocarbons from the sewage effluent discharging into the

river, suggesting that this may be the source of the hydrocarbons in the water and sediment.

Nitta (1972) reported on fish tainting in Osaka Harbour, Japan caused by a high oil content in the bottom mud resulting from the disposal of oil by ships. Further Japanese occurrences of fish tainting caused by industrial petroleum wastes were reported by Ogata and Miyake (1973). Offensive-smelling fish were caught in the sea facing the petroleum and petrochemical industries of the Mizushima district near Okayama.

Mackie et al. (1972) reported that trout caught eleven days after a spill of diesel fuel oil in Northern Ireland were found to smell and taste like fuel oil. A comparison of chromatograms showed that a large number of aliphatic saturated hydrocarbons and possibly some of the aromatics present in the diesel oil were also present in the hydrocarbon fraction isolated from the flesh of the tainted fish.

Although numerous shipping accidents have resulted in tainting, the best documented cases are the *Torrey Canyon* and *Amoco Cadiz* incidents where shellfish and finfish contamination was reported (Tidmarsh and Ackman 1986). However, in the *Torrey Canyon* incident it was believed that tainting was caused by the dispersants used, rather than the Kuwait crude oil spilled, since these dispersants consisted of surfactants dissolved in light refined oils (GESAMP 1977).

In 1973, a spill of 2,200 tonnes of diesel oil occurred after a tanker was grounded near Finnsnes in the north of Norway. Subsequent reports of oil tainting in the local fish were substantiated by organoleptic testing and Gas Chromatography / Mass Spectrometry (GC/MS) analysis of the fish flesh (Palmork and Wilhelmsen 1974, cited in GESAMP 1977).

Tidmarsh and Ackman (1986) stated that the only recent documented case of fish tainting occurring as a result of blowouts associated with offshore oil and gas activities was the Funiwa 5 blowout in the Niger Delta. No further documentation was provided on this incident.

2.2 POTENTIAL FISH TAINTING COMPOUNDS

2.2.1 Difficulties with Identifying Tainting Compounds

The assessment of the specific compounds responsible for fish tainting problems has been plagued by many difficulties. These may be summarized as follows:

1. Tainting can be caused by concentrations of volatile compounds that are at the lower limits of analytical detection.
2. The chemical analyses used to identify many of the potential tainting compounds are highly specialized and often cannot be used to unequivocally identify the compounds imparting taste and/or odours to fish flesh.
3. The metabolic processes of the fish may alter the chemical characteristics of contaminants, making it difficult to identify, by chemical analytical techniques, compounds in crude oil or condensates that exactly match hydrocarbons isolated from fish deemed to be tainted.
4. The specific source of the taint is difficult to determine, as fish are migratory and tainting may occur from biological as well as anthropogenic inputs.

5. The assessment of taint is a subjective measurement that is difficult to treat quantitatively. The degree of taint as assessed by sensory perception is based on individual experience and preference. Sensory evaluation procedures for assessing taint are dependent on ability of the panelists, their familiarity with the medium and tainting substances, the degree and type of training, and the sensory evaluation method used.
6. There is presently no defined standard procedure for the assessment of petroleum taint in fish flesh.

Despite these difficulties, the published literature yields considerable information that is useful for identifying specific oil and petroleum hydrocarbons with tainting potential. However, there is very little published information on the tainting compounds specific to oil sands tailings ponds.

2.2.2 Tainting Compounds Identified

Exhaustive analysis by Birkholz et al. (1987) of the water soluble extract obtained from an oil sample collected from the Suncor wastewater pond during the 1981-82 plant upset identified and confirmed the presence of alkylated benzenes, alkylated benzothiophenes and alkylated dibenzothiophenes. Compounds which were identified, but not taken to ultimate confirmation included alkylated furans, indans, quinolines and polynuclear aromatic hydrocarbons.

Wellington Environmental Consultants (1983a, 1983b) conducted a series of analyses on tainted walleye taken from the Athabasca River during the 1981-82 upset. Their initial work provided approximate quantitative estimates of whole body hydrocarbon contaminant levels, finding 40 to 150 ppb of aliphatic

hydrocarbons, 50 to 1000 ppb of polynuclear aromatic hydrocarbons and 30 to 1500 ppb of polynuclear aromatic sulphur heterocycles. Compounds that were subsequently specifically identified by GC/MS included dimethylbenzenes, tetramethylbenzenes and dimethylnaphthalenes.

Specific compounds in petroleum refinery effluents and oil-related products that are readily accumulated by fish and other aquatic organisms, and which have been linked to fish tainting incidents, may provide further evidence on potential tainting compounds from oil sands effluents.

Based on a review of the available literature, GESAMP (1977) concluded that the principal components of crude and refined oil causing tainting included the phenols, dibenzothiophenes, naphthenic acids, mercaptans, tetradecanes and methylated naphthalenes. Ogata and Miyake (1973, 1975), Ogata and Ogura (1976) and Ogata et al. (1987) identified toluene as the primary compound contributing to objectionable odours in fish exposed to petroleum industrial wastes. The average concentration of toluene in eels was 2.4 times that in the water (Ogata and Miyake 1973). The aromatic hydrocarbons, particularly benzene and o-, m- and p-xylene, were also implicated as contributors to the offensive odour. When the ratio of toluene concentration in eel flesh to that in water was expressed as an index of 100, the indices of m- or p-xylene, o-xylene and benzene were 28.7, 25.6 and 15.0, respectively (Ogata and Miyake 1975).

Roubal et al. (1977) found that when coho salmon were exposed to a dilute water soluble fraction of Prudhoe Bay oil for five weeks, they accumulated the more highly alkylated benzenes and naphthalenes in muscle tissue faster than the less-substituted aromatics. C_4- and C_5-benzenes and 2-methylnaphthalene had the highest bioconcentration factors of 458.3 and 140, respectively. Roubal et al.

(1978) found a similar pattern of differential accumulation of benzenes and naphthalenes in starry flounder. Fish exposed to 1 ppm of the water soluble fraction of Prudhoe Bay oil were observed to accumulate 9000 times the concentration of C_4- and C_5-substituted alkylated benzenes present in the surrounding waters.

Woodward et al. (1981) exposed trout to various concentrations of crude oil and concluded that the alkylated benzenes were the aromatic hydrocarbons which fish accumulated to the greatest extent. Woodward et al. (1983) found alkylated naphthalenes to be the dominant component of the aromatic fraction of a refined oil. Cutthroat trout exposed to 39 ppb of total oil accumulated 2.7 ppm of naphthalene.

Shipton et al. (1970) positively identified n-tetradecane, naphthalene and 2-methylnaphthalene, and with less certainty, a number of other benzene and naphthalene derivatives, in the tainted flesh of Australian mullet. These compounds were also identified in a commercial sample of kerosene.

Rossi et al. (1976) stated that naphthalene, methylnaphthalene and dimethylnaphthalene are the major high boiling aromatic hydrocarbons that transfer from oils into the water column, and consequently are more available for contact and uptake by aquatic organisms. Naphthalenes are also readily absorbed by aquatic organisms, but only slowly depurated. Gruenfeld and Frank (1977) suggested the use of naphthalenes and substituted naphthalenes as possible indicator parameters for the determination of petroleum incorporation into aquatic organisms because of their enhanced availability, rapid uptake and slow depuration.

Oysters exposed to an experimental spill of No. 2 fuel oil accumulated higher concentrations of the alkylated naphthalenes than the other aromatics and alkanes. These compounds were also retained longer in the tissue when the oysters

were returned to oil-free water (Neff et al. 1976a; Bieri and Stamoudis 1977). Although similar exposures using mummichogs (a marine fish) resulted in the same differential accumulation, the fish accumulated substantially higher concentrations of the compounds than did the oysters. The mummichogs also tended to retain the compounds in their tissues longer than the oysters (Bieri et al. 1977, cited in Neff 1979).

Clams exposed for 24 hours to the water soluble fraction of No. 2 fuel oil (total dissolved hydrocarbons of 6.28 ppm) accumulated 13.6 ppm total naphthalenes in their tissues. Methyl- and dimethylnaphthalenes were the forms reaching the highest concentrations (Neff et al. 1976a)

These results were corroborated by Stainken (1977, 1978), who found that monomethyl, dimethyl, and trimethylnaphthalene isomers were the principal compounds accumulated and retained by soft-shell clams exposed to No. 2 fuel oil-in-water emulsions under simulated winter (4°C) conditions. The dimethyl and trimethylnaphthalenes remained in the tissue after a two week depuration period.

Tatem (1977) also found that the methylated naphthalenes are the petroleum hydrocarbons which are accumulated and retained to the greatest extent by organisms exposed to oil-seawater mixtures. Naphthalene, methylnaphthalenes and dimethylnaphthalenes were rapidly accumulated by grass shrimp exposed to the water soluble fraction of No. 2 fuel oil. After 6 hours of exposure, tissue levels of methylnaphthalene were 150 times greater than the water levels.

Melancon and Lech (1978, 1979) studied the accumulation and elimination of naphthalene and 2-methylnaphthalene in fingerling rainbow trout. They found maximum tissue levels of from 40 to 300 times the water concentration after four weeks exposure in a continuous-flow delivery system.

Ogata et al. (1979) exposed eels and short-necked clams to a crude oil suspension. GC/MS analysis of the eel flesh revealed the presence of 1-methyl, 2-methyl, dimethyl, trimethylnaphthalenes and dibenzothiophene. Analysis of the soft body of the clams showed dimethylnaphthalene, trimethylnaphthalene, dibenzothiophene, and monomethyl and dimethyldibenzothiophenes. Ogata and Miyake (1978, 1980) found that eels exposed to crude oil exhibited flesh contamination by alkyl derivatives of benzothiophene and dibenzothiophene. Ogata and Fujisawa (1983) found accumulation of alkylated dibenzothiophenes (C_1 - C_3) to tissue concentrations of 6.71 ppb in oysters and 9.11 ppb in mussels caught in the Sea of Japan. On the basis of these results, Ogata et al. (1977, 1979) and Ogata and Miyake (1980) suggested the use of organic sulphur compounds as a marker of oil pollution in fish and shellfish because trace amounts can be relatively easily determined analytically.

Paasivarta et al. (1981) and Sinkkonen (1982) also measured a range of parts per million concentrations of alkyl dibenzothiophenes in fish and mussel from the Baltic. They found a good correlation between total oil residues and residues of dimethyldibenzothiophenes.

Phenolic compounds are common components of petroleum wastewaters, and have been implicated in both water and fish tainting problems (U.S. EPA 1973). Bandt (1955, 1958, cited in Alabaster and Lloyd 1982) found that dimethylphenols and other constituents of phenolic wastes, including naphthols and quinols, tainted bream and common carp at concentrations between 0.5 and 5.0 mg/L. However, Albersmeyer and Erichsen (Mann 1965, cited in Côté 1976) suggested that the taste change was not due to the accumulation of phenols in the fish tissue, but rather to the non-phenolic substances, such as aromatic and aliphatic

hydrocarbons, accompanying the phenols in waste water. Ruchhoft (1954, cited by Côté 1976) conducted threshold odour tests of receiving waters for refinery effluents and found that the aliphatic and aromatic hydrocarbons and sulphur heterocycles were of greater significance in causing odour than were the refinery-related phenolic materials (non-chlorinated phenols).

2.2.3 Tainting Compounds Selected for Study

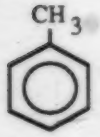
With this background, the methylated naphthalenes, methylated benzenes, sulphur heterocycles, and the phenols were selected as representative groups of compounds present in oil sands wastewaters with the potential for fish tainting. The specific compounds selected for sensory evaluation are summarized in Figure 2.

Walleye (*Stizostedium vitreum*) was chosen as the fish medium for these investigations because of its importance in the commercial, domestic and recreational fisheries in the Athabasca River north of Fort McMurray (Wallace and McCart 1984). Walleye flesh spiked with the selected compounds was screened for tainting intensity. The compounds detected by the panelists at the lowest levels were further evaluated for detection threshold levels.

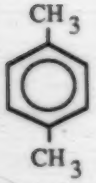
2.3 THRESHOLD VALUES FOR FISH TAINTING COMPOUNDS

The determination of threshold detection values for the compounds selected for sensory evaluation requires that the appropriate range of concentrations be presented to the sensory panel. Published threshold values in water and fish tissue for these compounds provide a basis for determining ranges for preliminary testing.

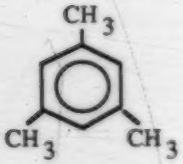
Alkylated Benzenes



Toluene



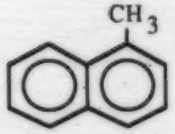
p-Xylene



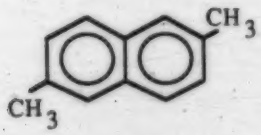
Mesitylene

Alkylated Naphthalenes

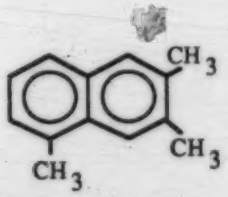
Naphthalene



1-Methylnaphthalene



2,6-Dimethylnaphthalene



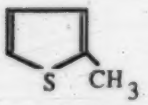
2,3,5-Trimethylnaphthalene

Figure 2. Compounds selected for sensory threshold evaluation

continued . . .

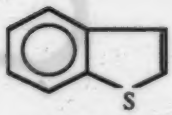
Alklyated Thiophenes

Thiophene

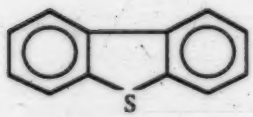


2-Methylthiophene

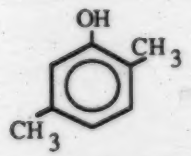
Other Compounds



Benzothiophene



Dibenzothiophene



2,5-Dimethylphenol

Figure 2. Concluded.

There have been very few studies on the levels of oil components required to cause threshold taint levels in fish tissue. This is further complicated because values may be expressed as either tissue concentrations or the corresponding water concentration required to produce the threshold taint. It is often unclear in the literature how the values are expressed.

Threshold detection values in water for the compounds of concern in this study are more common. However, the correlation of these values with the corresponding fish tainting values is not clear. Persson (1984) reported on 57 individual chemical compounds, several mixtures of compounds and several types of wastewaters that have been shown to cause off-flavour of fish under laboratory conditions. He concluded that for 61.4% of the compounds tested, concentrations impairing the flavour of fish were higher than the corresponding threshold odour concentration (TOC) in water. However, he also found that phenols as a group seemed to cause off-flavours in fish at concentrations lower than their TOC. He thus concluded that the aroma of water is a poor indicator of the flavour of fish living in it.

Persson's conclusions should be qualified because many of the literature values used in his review are dated pre-1960, before modern analytical techniques such as gas chromatography were in widespread use. These data may not, therefore, provide a reliable basis for determining, or comparing, threshold concentrations.

Preliminary range estimates for spiking the fish tissue were based on a combination of known literature tainting thresholds in fish tissue (Table 1), tainting threshold values of exposure waters (Table 2) and threshold odour concentrations in water (Table 3). When it was necessary to rely solely on the latter values, the

Table 1. Taste threshold concentrations in fish tissue reported in the literature for the compounds selected for threshold evaluation.

Compound	Taste Threshold Concentration in Fish Tissue (mg/kg)	Species	Reference
Toluene	200	Scallop	Motohiro and Iseya 1976 [1]
Xylene	100	Scallop	Motohiro and Iseya 1976 [1]

1 As cited in Motohiro (1983)

Table 2. Threshold odour concentrations in fish (expressed as exposure concentration in water) reported in the literature for the compounds selected for threshold evaluation.

Compound	Threshold Odour Concentration (in exposure water) (mg/L)	Species	Reference
Naphthalene	1 - 3.4	Roach, tench, crucian carp	Bandt 1955 [1]
			Albersmeyer and Erichsen 1959 [1]
1-Methylnaphthalene	2.6	Tench, crucian carp	Albersmeyer and Erichsen 1959 [1]
Toluene	0.25 - 50	Yellow perch	Mann 1953 [1]
			Winston 1959 [1]
Xylene	0.3	?	OHM-TADS 1981 [2]
2,5-Dimethylphenol	1.0	Roach	Bandt 1955 [1]

1 As cited in Persson (1984)

2 As cited in Trudel (1986)

Table 3. Odour threshold values in water reported in the literature for the compounds selected for threshold evaluation.

Compound	Threshold Odour Concentration (in water) (mg/L)	Reference
Naphthalene	0.5	Hollutta 1960 [1]
	6.80	Rosen et al. 1963
	0.001	Koppe 1965 [1]
	0.005	Zoeteman et al. 1971 [1]
1-Methylnaphthalene	0.02	Lillard et al. 1975
	0.0075	de Grunt 1975 [1]
2,6-Dimethylnaphthalene	0.010	Brady 1968 [1]
	0.0067	Seifert et al.1975 [2]
Toluene	1.0	Zoeteman et al. 1971 [1]
	0.024	Alexander et al. 1982
p-Xylene	0.53	Rosen et al. 1962
Mesitylene	0.027	Baker 1963
	0.5	Zoeteman et al. 1971 [1]
	0.003	de Grunt 1975 [1]

1 Cited in van Gemert and Nettenbreijer (1977)

2 Cited in Fazzalari (1978)

lowest reported water concentration was increased by at least one order of magnitude in setting fish-spiking concentrations, in accordance with Persson's findings.

For general reference, threshold values for whole oil were also used. GESAMP (1982) reported a threshold of 10 to 30 ppm in fish tissue spiked with a North Sea crude oil, with an upper limit of 200 to 300 ppm, beyond which no further increases were perceived by a trained taste panel. Threshold levels of 5 ppm gas oil in spiked mussel tissues, and 4 to 12 ppm extractables from diesel oil in lobsters were also reported. Kerhoff (1974, cited in Connell and Miller 1981) found oily taste thresholds at hydrocarbons concentrations of greater than or equal to 5 ppm in blue mussel tissue exposed in an estuarine gas oil spill.

Nitta (1972) reported on experiments done at Yokkaichi in 1963 which showed that saurel kept in 0.01 ppm of oil in sea water took on a slight odour in 24 hours. Mackerel took on an odour in sea water containing approximately 0.05 ppm of oil.

Alexander et al. (1982) reported an odour threshold value of 0.0008 mg/L and a taste threshold value of 0.024 mg/L for No. 2 fuel oil in water.

2.4 PHYSICOCHEMICAL PROPERTIES OF THE COMPOUNDS

The behavior of organic chemicals in the environment can be directly related to certain physical and chemical properties of the compounds. Of particular concern to this study are those properties that affect, and can thus be used to predict, the environmental fate of the compound in terms of its uptake and bioconcentration in the fish tissue. Also of concern are the factors that contribute to the relative volatility of the compound, and thus the ease with which it can be organoleptically detected.

2.4.1 Octanol-Water Partition Coefficients

The octanol-water partition coefficient (written as K_{ow}, P or P_{ow}) is defined as the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of an equilibrium two-phase octanol/water system. This coefficient is important in studies of the environmental fate of organic chemicals because it is related to water solubility, soil/sediment adsorption coefficients and bioconcentration factors for aquatic life (Lyman 1982).

Values of K_{ow} represent the tendency of the chemical to partition itself between an organic phase, such as fish tissue, and an aqueous phase. Neff et al. (1976b, cited in Neff 1979) suggested that the binding of petroleum hydrocarbons to tissue lipids was by hydrophobic interactions, rather than covalent bonding, and was dependent on the octanol-water partitioning of the hydrocarbons.

Log K_{ow} values for the compounds selected for threshold evaluation in this study are given in Table 4. The logarithmic form is normally used for the estimation of other physical parameters. These values are used in the following sections to estimate water solubility where these values have not been experimentally determined. They are also used to estimate bioconcentration factors.

2.4.2 Volatility

The organoleptic sensation experienced on consuming petroleum hydrocarbons is primarily due to the volatile components present. These components generate a flavour aroma within the food which is described as a taint by taste panels (Connell and Miller 1981).

The factors that control volatilization are the solubility, molecular weight and vapour pressure of the chemical. Henry's Law Constant relates the

Table 4. Octanol-water partition coefficients at 25°C for the compounds selected for threshold evaluation.

Compound	log K_{ow}	Reference
Naphthalene	3.35	Mackay and Shiu (1977)
1-Methylnaphthalene	3.86	Mackay and Shiu (1977)
2,6-Dimethylnaphthalene	4.38	Mackay and Shiu (1977)
2,3,5-Trimethylnaphthalene	4.90	Mackay and Shiu (1977) [1]
Toluene	2.69	Verscheuren (1983) [2]
p-Xylene	3.15	Verscheuren (1983)
Mesitylene	N/A	
Thiophene	1.6	Vassilaros et al. (1982a) [3]
2-Methylthiophene	N/A	
Benzothiophene	3.1	Vassilaros et al. (1982a) [3]
Dibenzothiophene	4.4	Vassilaros et al. (1982a) [3]
2,5-Dimethylphenol	2.35	Verscheuren (1983) [4]

1 Value is for 1,4,5-trimethylnaphthalene.

2 Value is for 20°C.

3 Cited in Hrudey and Nelson (1987).

4 Value is for 3,5-dimethylphenol.

N/A Value is Not Available in the literature

concentration of a compound in the gas phase to its concentration in the liquid phase, and is calculated as the ratio of vapour pressure over water solubility (Thomas 1982). This constant thus compensates for the conflicting potentials of a compound to evaporate and to remain dissolved in the water. Table 5 summarizes the molecular weights, vapour pressures, water solubilities and Henry's Law constants for each of the compounds selected for testing.

Figure 3 illustrates the volatility characteristics associated with various ranges of Henry's Law Constant. With the exception of 2,5-dimethylphenol, which is only slightly volatile, all the compounds have a Henry's Law Constant of greater than 10^{-4} atm-m^3/mole, and are thus significantly volatile in all waters.

2.4.3 Bioconcentration Factors

Bioconcentration factors (BCF) are a measure of the potential of a chemical to accumulate in the tissues of aquatic organisms, and are useful in assessing the overall environmental hazard of many chemicals (Bishop and Maki 1980). The bioconcentration factor relates the concentration of a chemical in ambient media (usually water) to the concentration of the chemical in the organism, and is defined as:

$$BCF = \frac{\text{Concentration of chemical at equilibrium in organism (wet weight)}}{\text{Mean concentration of chemical in water}}$$

Bioconcentration is based on the assumption that uptake from water across external membranous surfaces is the chief source of the compound that is concentrated in the organism (Bysshe 1982).

BCF's may be measured experimentally either by determining uptake and depuration rates of an aquatic organism, or by exposing the organism to a constant concentration of the chemical and measuring the chemical residue concentration in

Table 5. Summary of the physical properties affecting volatility for each compound tested.

Compound	Molecular Weight	Vapour Pressure (torr) at 25°C	Water Solubility (mg/L) at 25°C	Henry's Law Constant ($atm \cdot m^3/mole$)
Naphthalene	128.17	0.23 [1]	33 [1]	1.18×10^{-3}
1-Methylnaphthalene	142.20	0.05 [2]	28.0 [3]	3.34×10^{-4}
2,6-Dimethylnaphthalene	156.23	0.015 [4]	2.02 [3]	1.53×10^{-3}
2,3,5-Trimethylnaphthalene	170.25	0.010 [5]	2.03 [6]	1.10×10^{-3}
Toluene	92.14	28.4 [1]	515 [1]	6.68×10^{-3}
p-Xylene	106.17	8.0 [7]	198 [7]	5.64×10^{-3}
Mesitylene (at 15.5°C)	120.19	2.42 [2]	20 [8]	1.91×10^{-2}
Thiophene	84.14	56.68 [4]	1430 [7]	4.39×10^{-3}
2-Methylthiophene	98.16	24.89 [4]	N/A	N/A
Benzothiophene	134.20	N/A	N/A	2.27×10^{-4} [9]
Dibenzothiophene	184.26	N/A	N/A	4.40×10^{-4} [9]
2,5-Dimethylphenol	122.17	0.12 [10]	7867 [11]	2.45×10^{-6}

1 Mackay and Leinonen (1975)

2 Dean (1985). Note: 25°C is outside the temperature range of the data used in evaluating the coefficients of the vapour pressure equation.

3 Mackay and Shiu (1977)

4 Dean (1985)

5 Chao et al. (1983). Note: value is estimated based on given value for 127°C.

6 Mackay and Shiu (1977). Value is for 1,4,5-trimethylnaphthalene.

7 Vassilaros et al. (1982a, cited in Hrudey and Nelson 1987)

8 American Petroleum Institute (1969)

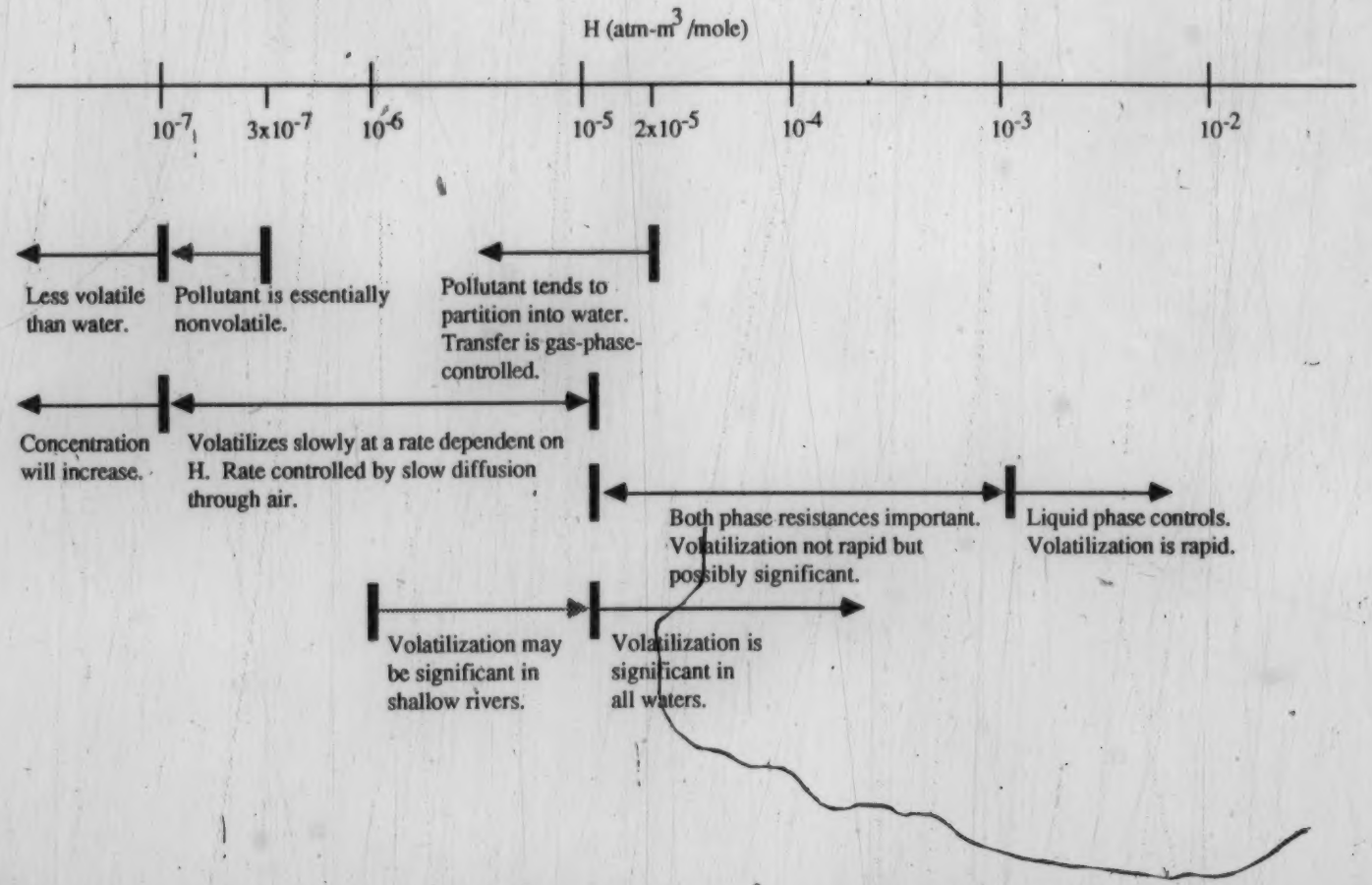
9 Thomas (1982)

10 Estimated as per Grain (1982)

11 Banerjee et al. (1980). Value is for 2,4-dimethylphenol.

N/A Value is Not Available in the literature

Figure 3. Volatility characteristics associated with various ranges of Henry's Law Constant (modified from Thomas 1982).


H (atm-m3 /mole)
10-7
3x10-7
10-6
10-5
2x10-5
10-4
10-3
10-2
Less volatile than water.
Pollutant is essentially nonvolatile.
Pollutant tends to partition into water. Transfer is gas-phase-controlled.
Concentration will increase.
Volatilizes slowly at a rate dependent on H. Rate controlled by slow diffusion through air.
Both phase resistances important. Volatilization not rapid but possibly significant.
Liquid phase controls. Volatilization is rapid.
Volatilization may be significant in shallow rivers.
Volatilization is significant in all waters.

the organism after equilibrium is reached. If the BCF has not been measured or is not readily available, it can be estimated based on one of several existing regression equations developed through laboratory experimentation. Veith et al. (1980) developed a correlation with the octanol-water partition coefficient, which has been recommended for BCF estimation (Bysshe 1982):

$$\log BCF = 0.76 \log K_{ow} - 0.23$$

This equation is based on the results of laboratory experiments by several investigators with a variety of fish species and 84 different organic chemicals.

Table 6 compares values of BCF measured in the laboratory for selected aquatic species with estimates derived from correlations based on K_{ow}. This table illustrates the large discrepancies which are common between estimated and measured values, and between measured values for different species. These observed differences are due to the variability inherent in biological responses, differences in exposure medium and factors responsible for measurement inaccuracy. A BCF value may vary considerably between species and between life stages for a single species (Kenaga and Goring 1980). Neff and Anderson (1981) suggested that the difference in BCF's reported for naphthalene in the clam *Rangia cuneata* may imply that bioaccumulation of a particular hydrocarbon is influenced by the presence of other aromatic hydrocarbons in the exposure medium. The lower value of 2.3 was for uptake from the water soluble fraction of No. 2 fuel oil, whereas the higher value of 6.1 was for uptake from water containing only naphthalene. Finally, measured BCF's are based on variable, and often unreported, times of exposure. Errors in measuring K_{ow}, such as variation in measurement methods, interpretation of results, the use of estimates and test

Table 6. Estimated and measured bioconcentration factors for the compounds selected for threshold evaluation .

Compound	BCF (Estimated)	BCF (Measured)		
		BCF	Species	Reference
Naphthalene	207	426	Fathead minnow	Veith et al. 1980
		60	Sheep's-head minnow	Anderson et al. 1974 [1]
		50	*Daphnia magna*	Eastmond et al. 1984
		131	*Daphnia pulex*	Southworth et al. 1978
		60	Coho salmon	Roubal et al. 1978
		240	Starry flounder	Roubal et al. 1978
		6.1	Clam *Rangia cuneata*	Neff et al. 1976b [1]
		2.3	Clam *Rangia cuneata*	Neff et al. 1976a
1-Methylnaphthalene	505	210	Sheep's-head minnow	Anderson et al. 1974 [1]
		100	Coho salmon	Roubal et al. 1978
		330	Starry flounder	Roubal et al. 1978
		8.5	Clam *Rangia cuneata*	Neff et al. 1976a
2,6-Dimethylnaphthalene	1260	17.1	Clam *Rangia cuneata*	Neff et al. 1976a
2,3,5-Trimethylnaphthalene	3120	26.7	Clam *Rangia cuneata*	Neff et al. 1976a

continued . . .

Table 6. Concluded.

Compound	BCF (Estimated)	BCF (Measured)		
		BCF	Species	Reference
Toluene	65			
p-Xylene	146			
Thiophene	9			
Benzothiophene	133	750	*Daphnia magna*	Eastmond et al. 1984
Dibenzothiophene	1300	600	*Daphnia magna*	Eastmond et al. 1984
		1321	Oyster	Ogata and Fujisawa 1983
		1320	Mussell	Ogata and Fujisawa 1983
2,5-Dimethylphenol	35	150	Bluegill sunfish	Veith et al. 1980

1 As cited in Neff and Anderson (1981)

conditions (such as pH and temperature), are also responsible for some of the discrepancies observed (Bysshe 1982).

For the purposes of this study, a relative comparison of BCF's for the compounds selected for threshold evaluation will be based on the estimated values because these values are derived on a common basis. The potential of the alkylated naphthalenes and benzenes to accumulate in fish flesh increases by more than a factor of two with the addition of each subsequent methyl group. However, the alkylated naphthalenes have much higher propensity for accumulation. This is supported by the findings of Roubal et al. (1978) and Neff et al. (1976a), as reported earlier in this review.

Thiophene and dimethylphenol have a very low potential for bioaccumulation. The BCF increases dramatically for dibenzothiophene from benzothiophene.

2.5 SENSORY THRESHOLD EVALUATION METHODS

Sensory evaluation is the measurement of physical properties using psychological techniques. Although sensory evaluation methods can be applied with all the human senses, they most commonly involve using the senses of taste and odour to establish differences of a product against a known standard or control, or to establish preferences among a group of products (A.S.T.M. 1968a).

Sensory ratings or comparisons are usually conducted using trained or untrained people assembled in a taste panel. When sensory evaluation is used for precise measurements, such as threshold determinations, the sensory panel is essentially a laboratory instrument, and as such, should be calibrated for accuracy and precision. Panelists should have a known sensitivity, and be selected and

trained to demonstrate this sensitivity consistently for the required sensory tasks (Vaisey Genser 1977).

The choice of a particular sensory evaluation method depends on factors such as the number of samples to be evaluated, the quantity of product available, the information desired, and the degree of panel training. No singular method is used for threshold determinations, although the need for a standardized, reproducible method for threshold determinations has long been recognized (Baker 1963). The two most common methods used in the determination of absolute threshold values are variations of the paired comparison test and the triangle test .

A.S.T.M. (1968a) outlined two paired comparison methods for determining the minimum detectable level or concentration of a substance, defined as the absolute threshold. The Constant Stimulus Differences method involves pairing each sample with a standard of zero concentration, and presenting the samples in random order. The panelist judges which sample in each pair is stronger. This method was judged to be unacceptable for determining thresholds of volatile compounds because it involves the preparation and evaluation of a large number of samples, and because the use of strong-smelling compounds would dictate presenting the samples in order of ascending concentration, rather than in random order.

In the Method of Limits, the subject is trained to recognize the specific attribute under investigation. The samples are presented in order of physical concentration (either ascending or descending), and the panelist judges the presence or absence of the designated taste and odour. Blank samples of zero concentration may be randomly interspersed throughout the series to overcome the expectation function of the panelists. Rosen et al. (1962) proposed a modification of this

method called the Consistent Series Threshold Method. This is an ascending concentration series in which each sample is compared to a reference sample of zero concentration. The method is further modified from the Method of Limits by requiring the panelists to judge only whether there is a difference between the sample and the reference, and not whether a specific taste or odour exists.

In the triangle test, three samples are presented to the panelist. Two of the samples are the same, and one is different. The panelist judges which sample he believes is different. This is a forced choice method; the panelist cannot abstain from a decision even if he does not detect any differences between the samples (A.S.T.M. 1968a). This test is similar to A.S.T.M. standard method D-1292-65 (1968b), and has been recommended as the test that would be the most useful and reliable tool in identifying possible tainting in fish (Tidmarsh and Ackman 1986). Cohen et al. (1960) used a Modified Triangle Test for threshold determinations, in which the panelists were further required to state whether the odd samples contained the chemical attribute under investigation.

Each test has advantages and disadvantages. The paired comparison test would involve the preparation and evaluation of fewer samples than the triangle test. However, in any paired comparison test there is a 50% chance of guessing whether a sample is tainted or untainted. This probability is somewhat reduced by using interspersed blank samples as an incentive to honestly evaluate a sample. The triangle test has only a 33% chance of guessing, but involves the evaluation of considerably more samples, and thus introduces the possibility of panelist fatigue. It also requires more tainted product and increased preparation time for the investigator.

3. METHODS

3.1 SELECTION OF SENSORY THRESHOLD EVALUATION METHOD

To decide which test was more sensitive for determining the threshold values of the selected chemicals in fish tissue, preliminary tests were conducted to compare the threshold values for benzothiophene obtained using the Consistent Series Test and the Modified Triangle Test.

3.1.1 Sample Preparation

Fresh whitefish, caught in Alberta, were used in the first two replications of the experiment. Frozen whitefish from the Freshwater Fish Marketing Corporation in Winnipeg were substituted in the third and fourth replications, as the fresh fish was unavailable.

Approximately 2 kg of fish were used in each replication. The fish were skinned and filleted, and the flesh of all fish minced together in a Braun Multiquick Food Chopper. This eliminated any variability in either the natural odour, or in any existing concentration of odour-causing contaminants, between individual fish.

There are no reported threshold values for benzothiophene in the literature for either fish flesh or water. The concentration range for these tests was therefore determined from existing literature on threshold values of naphthalene in water (which is similar to benzothiophene in odour and chemical properties) and on the basis of a preliminary run using a wide range of concentrations, ranging from 0.031 to 100.0 mg/kg. Based on the sensory response of the panelists, it was decided to use a range of 0.031 to 1.000 mg/kg. Previous research has determined that the accuracy and reproducibility of panelists in threshold odour determination

corresponds to a dilution series based on a factor of two (Rosen et al. 1962; Hamilton 1978). This means that dividing a given odour sample in half by untainted medium in each step of the dilution is approximately equal to the human sensory perception of minimal difference in odour intensity. The concentration levels of benzothiophene are based on increasing each successive sample by a factor of two, within the determined appropriate range, as shown in Table 7.

The low concentrations of benzothiophene being added to the fish tissue, and the crystalline nature of the compound at room temperature, posed a problem in the addition of the substance to the fish tissue. The possibility of using an ethanol carrier to insure adequate distribution of the compound throughout the tissue was considered (Persson 1984). However, because of the relatively untrained nature of the panel, it was decided that this would hinder odour perception of the benzothiophene. Instead, the benzothiophene was added to slightly warm (approximately 35°C) fish tissue as it was being minced. The melting point of benzothiophene is 31°C. Consequently, the crystals were liquified before actually coming into contact with the fish. For the purposes of this phase of the study only, it was assumed that this procedure resulted in uniform distribution of the chemical throughout the minced fish.

Using this spiking procedure, the most accurate method of obtaining the low concentrations required for the threshold tests was to prepare a highly concentrated sample of 100 mg/kg, and progressively dilute it with untainted fish to the required concentrations. The initial dosage of benzothiophene was weighed on a Mettler AE 163 analytical balance.

Five grams of each concentration level of "tainted" fish and the "blank" fish were added to the appropriate number of 15 mL scintillation vials required for each

Table 7. Concentration levels of benzothiophene used in odour threshold determinations.

Dilution Number	Benzothiophene Concentration (mg/kg)
1	0.031
2	0.063
3	0.125
4	0.250
5	0.500
6	1.000

test. The samples were weighed on a Sartorius 2253 Balance. The number of samples of each concentration was determined prior to each test and based on the random presentation order assigned to that replication. Each sample vial was coded on both the lid and the bottle by three-digit random numbers.

3.1.2 Testing Procedures

Both tests were conducted in each replication of the experiment. A total of four replications were conducted between November 5 to 27, 1985. As the two tests involved a large number of individual evaluations (a total of 27), the possibility of sensory fatigue, and its subsequent bias to the first set of samples presented, was recognized. The order of the presentation of each test to the panelists was reversed for every replication to eliminate the effect of this bias on the testing results.

3.1.2.1 Consistent Series Threshold Method As described in Section 2.5, the Consistent Series Threshold Method (Rosen et al. 1962) is an ascending concentration series in which each sample is compared to a reference "blank" sample. The test is thus a form of the paired comparison testing method, with blank samples randomly interspersed throughout the series (A.S.T.M. 1968a)

Ten samples were presented to each panelist for each replication of the test. Six of the samples contained the different concentrations of benzothiophene. Three "blank" untainted samples were inserted randomly between the tainted samples. A reference "blank" sample was also included for comparison. The tainted samples were arranged in ascending order of concentration.

An example of the presentation of the samples to each panelist is illustrated in Table 8. Theoretically, there is a minimum concentration in the test series which the panelist will identify as having a detectable odour. All the following higher concentrations will then be detected, and the intervening blanks judged odourless. This minimum concentration is then judged to be the absolute threshold concentration. However, anomalous responses may occur, such as a low concentration or a blank being identified as positive and a higher concentration identified as negative. In this case, the threshold is designated as the lowest concentration after which no further anomalies occur. In the examples given in Table 8, the threshold concentration for Panelist A occurs at 0.25 mg/kg, whereas for Panelist B it occurs at 0.5 mg/kg.

The positions of the 'blanks" in the series were assigned randomly, and were different for each replication. Random three-digit codes were assigned to each of the samples. These codes were also different for each replication of the experiment.

The questionnaire used in the evaluations conducted by this method is given in Figure 4. The panelists were informed that the tainted samples were arranged in order of increasing concentration, but were randomly interspersed with untainted "blank" samples.

3.1.2.2 Modified Triangle Test The triangle test method was previously described in Section 2.5. The method used in these tests was modified in that the panelists were further required to state whether the odd samples were of tainted or untainted fish (Cohen et al. 1960).

Table 8. Sample presentation and evaluation using the Consistent Series Test.

Sample No.	Benzothiophene Concentration (mg/kg)	Panelist A	Panelist B
1	0.031	-	-
2	0.063	+	-
3	Blank	-	-
4	0.125	-	+
5	Blank	-	-
6	0.250	+	-
7	0.500	+	+
8	Blank	-	-
9	1.000	+	+

QUESTIONNAIRE FOR CONSISTENT SERIES TEST
ODOUR TEST

NAME _____ DATE _____

PRODUCT _____

Nine coded samples are provided. Some of these samples are tainted and some are untainted "blanks". The tainted samples are arranged in order of increasing concentration. An untainted "blank" sample is provided for comparison.

Test the samples separately and in the order indicated. Remove the lid from the jar and sniff the contents three times. Compare the sample with the "blank" and indicate if there is a difference in odour. Replace the lid, and repeat the procedure for the other samples. Rinse between each sample with the water provided.

Code	Is there a difference from the "blank"? (indicate yes or no)
____	_____
____	_____
____	_____
____	_____
____	_____
____	_____
____	_____
____	_____
____	_____

Figure 4. Questionnaire for the Consistent Series Test.

Six sets of three samples were presented to each panelist. Each set consisted of either one sample of tainted fish and two of untainted fish, or one sample of untainted fish and two of tainted fish. Each successive set contained a sample of an increased concentration of benzothiophene.

A sample test plan is illustrated in Table 9. As there were six concentrations to be tested, and six possible combinations of tainted and untainted samples, the design is completely balanced. The method for determining the detection threshold for each panelist is the same as that described for the Consistent Series Test.

The order of presentation of each combination was assigned randomly to the sample sets, and was different for each replication of the experiment. Random three-digit codes were assigned to each of the samples. The codes were different for each replication of the experiment.

The questionnaire used for this testing method is given in Figure 5.

3.1.3 Sample Presentation

The nine coded samples and the reference blank for the Consistent Series Test were presented to each panelist in one Corningware dish (1 L capacity). Due to the large number of samples (eighteen) in the Modified Triangle Test, it was necessary to place the first three sample sets (nine samples) in one dish and the remaining three sample sets in a second dish. The samples were heated to 40°C by adding sufficient water (approximately 250 mL) to the dish to totally submerse the portions of the vial containing the fish, and heating the dish in a Panasonic microwave oven (Model NE 7800) until the temperature of the water reached 40°C (approximately 1 minute at HIGH power). Heat was maintained in the samples throughout the sensory evaluation by keeping the samples in the water bath, and

Table 9. Sample test plan for the Modified Triangle Test. "A" represents a tainted sample and "B" represents an untainted sample.

Sample Set	Sample Order
1	AAB
2	ABA
3	BAA
4	BAB
5	ABB
6	BBA

QUESTIONNAIRE FOR TRIANGLE TEST
ODOUR TEST

NAME _____ DATE _____

PRODUCT _____

Six sets of three coded samples are provided. Each set should be evaluated separately, in the order listed. In each set, two of the three samples are identical, and the third is different. Test the samples separately and in the order indicated. Remove the lid from the jar and sniff the contents three times. Replace the lid, and repeat the procedure for the other samples. Check the odd sample and indicate whether the odd sample is tainted or untainted. Rinse between each sample with the water provided.

	Code	Check odd sample	Odd sample is: (check one)
SET #1	___	_____	Tainted _____
	___	_____	Untainted _____
	___	_____	
SET #2	___	_____	Tainted _____
	___	_____	Untainted _____
	___	_____	
SET #3	___	_____	Tainted _____
	___	_____	Untainted _____
	___	_____	
SET #4	___	_____	Tainted _____
	___	_____	Untainted _____
	___	_____	
SET #5	___	_____	Tainted _____
	___	_____	Untainted _____
	___	_____	
SET #6	___	_____	Tainted _____
	___	_____	Untainted _____
	___	_____	

Figure 5. Questionnaire for the Modified Triangle Test.

placing the dish on a 200 mm x 200 mm Salton warming element. The purpose of heating the samples was to improve odourant volatility. The 40°C temperature is the standard condition for odour tests recommended by A.S.T.M. (1968b), and is the temperature which panelists have assessed to be the most efficient for odour perception (Hamilton 1978).

The threshold odour evaluations took place in a special taste panel room designed to control background odours, room temperature, humidity and distracting activity that may interfere with the odour evaluation.

3.1.4 Panelists

The five panelists consisted of two females and three males, ranging in ages from 22 to 37 years. None of the panelists were smokers. Due to the time limitations of this part of the study, the standard selection procedure for panelists was not used. The primary criteria used in the selection of the panelists were availability, motivation and interest.

The training of the panelists was also restricted by the available time. A brief training session was held with the panel prior to the preliminary run of the tests. The panelists were briefed on the objective of the project, and the testing procedures to be used. A sample of the tainted fish, with a high concentration of benzothiophene (100 mg/kg) was also presented to the panelists to familiarize them with the odour of the benzothiophene in the whitefish tissue. The panelists received further training through the preliminary run of the experiment on November 4, 1985.

3.2 DETERMINATION OF THRESHOLD DETECTION VALUES

3.2.1 Ethics Committee Review

Prior to commencing the formation of a formal sensory panel for the determination of the threshold detection values, the proposed research plan was reviewed by an internal Civil Engineering ad hoc ethics committee. This review is standard procedure for all university research projects involving human participants. The purpose of the review is to protect the participants' safety and welfare by fully assessing the potential risks involved in undertaking the research.

The submission to the ethics committee included information on the background of the research, experts consulted for advice on experimental design, the proposed research procedure and data obtained from the RTECS (Registry for Toxic Effects of Chemical Substances) file showing the comparative toxicity of some of the chemicals to be used in the research. Additional information on the chemicals was provided by the Canadian Centre for Occupational Health and Safety. The known toxicity data on the chemicals showed no potential hazard to the panelists at the concentrations to be used in the research.

At the request of the ethics committee, the following procedures were adopted and actions were taken prior to commencement of this phase of the research to minimize the risks to the panelists:

1. A standard form was developed to provide a written record of all calculations for the addition of chemical compounds in the preparation of the spiked fish samples. These calculations were verified by a second party. Two people were always involved in the actual spiking of the fish.

2. A copy of all questionnaires and information material used in the selection of the prospective panelists, and copies of the waiver form signed by each were filed with the thesis supervisor immediately upon completion.

3. Prior to introduction of each new testing procedure the panelists as a group were thoroughly briefed by the investigator on the techniques to be employed. Comprehensive instructions on the testing procedure were also included on the sensory evaluation forms. The panelists were supervised to preclude any consumption of the fish. Any deviation from this policy was to be documented in writing.

4. On the advice of University Health Services, the following procedures were adopted in the event of fish samples being accidentally swallowed, or if one of the panelists developed an unforeseen physical reaction (such as an allergic reaction) during the course of the sensory evaluations:

   (a) In the event of any non-life threatening situation, the panelist was to be transported immediately to University Health Services. A list of the chemical compounds to be used in the tests was filed with Health Services to ensure that the procedures necessary to deal with any problems could be determined in advance of the tests.

   (b) In the case of any life-threatening reaction (such as shock or convulsions), an ambulance would be called to provide immediate life support function, and transport to the University of Alberta Hospital.

3.2.2 Preparation of Spiked Fish Samples

Frozen, whole medium-sized walleye from the Freshwater Fish Marketing Corporation were used. The fish were thawed in cold water, filleted and skinned. The flesh of several fish was minced together in a Moulinex (No. 588) Food Processor to eliminate any natural variability in the taste and odour between individual fish. The minced fish flesh was then frozen in vacuum sealed packages of the weights required for subsequent spiking.

The fish samples were prepared nine days prior to the first sensory evaluation. Sufficient samples were prepared at one time for three replications of the sensory evaluation. The time consuming nature of the sample preparation dictated this amount of advance preparation time to ensure continuity in the panel evaluations.

The spiked concentration ranges and intervals for each chemical compound were determined from existing literature and on the basis of preliminary tests for each compound using a wide range of concentrations. The final concentration levels used in the threshold determination for each compound were based on the determined appropriate range, and on increasing the concentration of each successive sample by a factor of two (as discussed in Section 3.1.1).

The procedure used in the comparison of sensory methods for spiking the chemical compound into the raw fish tissue was judged to be unacceptable for the purposes of detailed threshold evaluation because it did not adequately ensure accurate concentrations and homogeneity of mixing of the compound in the fish tissue. Therefore, for the actual threshold evaluations a concentrated solution was made up for each compound. Ethanol was used as the carrier solvent for naphthalene, 1-methylnaphthalene, benzothiophene (first test) and

dibenzothiophene. However, preliminary tests showed that the ethanol odour was interfering with the taste and odour identification for those compounds with higher threshold levels which required larger quantities of the spiking solution. Mineral oil (USP) was therefore used as a carrier solvent for the 2,6-dimethylnaphthalene, 2,3,5-trimethylnaphthalene, benzothiophene (second test), p-xylene and 2,5-dimethylphenol tests. Mineral oil was also used as a carrier for the preliminary tests done on thiophene, 2-methylthiophene, toluene and mesitylene.

The chemical compounds were weighed on a Mettler AE 163 analytical balance directly in the volumetric flasks used to prepare the stock solutions. To avoid measurement errors for stock solutions of extremely low concentrations, a solution of higher concentration was prepared and diluted to the required concentration.

During preparation of the samples, the fish was kept partially frozen to allow better mixing, and to minimize loss of the volatile spiking compounds. The fish was first cut into approximately 20 x 20 x 20 mm pieces, then ground in a Waring Model 5011 commercial blender to a powder consistency. The samples were spiked by pipetting the appropriate amount of the compound, in its solvent carrier solution, directly into the blender. All samples, including the blank and reference samples, were also spiked with an additional amount of solvent carrier equivalent to that present in the highest concentration to ensure that the presence of the carrier solvent remained a constant factor and did not influence the sensory evaluations. For extremely low concentrations, a microlitre syringe was used to measure the compound (in its carrier solvent), and this was added to the additional carrier solvent before addition to the fish. The entire weight of fish to be spiked at that concentration was spiked and blended together at the same time. Blending

consisted of mechanized blending for 20 seconds, followed by 50 strokes of manual mixing. This procedure was repeated four times for each spike.

The scintillation vials used to contain each sample in the preliminary methods selection study were expensive, difficult to prepare and provided only a small opening for odour perception. Therefore, folded foil packets, as described by Iredale and York (1976) were used for each sample in the threshold evaluations. Ten-gram aliquots (± 0.5 gm) were weighed on a Mettler PE 3600 electronic top-loading balance, and immediately wrapped in aluminum foil packets of approximately 80 mm x 40 mm folded dimensions. A three-digit code was marked on each packet with a wax pencil for identification during sensory analysis. The packets were then vacuum-sealed in polyethylene bags using a Decosonic (No. 828) Vacuum Bag Sealer. Four packets were sealed in each bag, and the bags frozen until prior to the actual evaluation. The three evaluations occurred nine, ten and twelve days, respectively, after the sample preparation.

Care was taken that the tissue was exposed to the air as little as possible during the spiking and weighing of the individual samples to minimize the loss of the volatile compounds. Because of the nature of the compounds being used, only glass and stainless steel instruments were used in the preparation of the samples. Soap was not used by the investigators preparing the samples either prior to or during the preparation procedure. If detergent or solvent (acetone) was necessary to remove the fish and chemical odours of the equipment following the spiking, it was followed by a minimum of ten rinses in distilled water. The Waring blender was dismantled and thoroughly cleaned between spikings of each compound to prevent contamination of the next set of samples.

3.2.3 Sensory Panel Screening

An initial group of 28 people were screened as potential sensory panelists. This group consisted of interested individuals who were non-smokers, liked fish and were available for the required tests. Panelists were recruited through a notice posted around the university, and through personal requests by the investigators. The three investigators who would be using the panel in their research also participated in the screening. Eleven males and seventeen females, ranging in age from 19 to 40 years of age were screened as potential panelists.

All potential panelists were requested to complete a personal history form (Appendix A) and to sign a waiver. Panelists were also instructed, both verbally and through written notification, to refrain from eating or drinking for at least an 30 minutes prior to the sensory tests, and to avoid the use of perfume, aftershave and scented soap on the days that panels were held. An information package explaining the nature of the study and the role of the sensory panelists was prepared and distributed to each potential panelist (Appendix A).

Motivation was provided through a small monetary remuneration for participating in the screening tests, with the prospects of a larger remuneration for participation as a final panelist, if selected.

Three screening tests were used to select the final panelists. The screening objectives were to eliminate people who were ageusic and anosmic (blind to taste and odours, respectively), and to evaluate individual discrimination ability and consistency of evaluation.

3.2.3.1 Taste Recognition Taste recognition screening tests were conducted July 14 and 17, 1986. Panelists were tested for their ability to recognize the basic tastes

of sweet, sour, bitter and salty. Suprathreshold concentration solutions of sucrose (sweet), citric acid (sour), quinine (bitter) and NaCl (salty) were prepared in distilled water. Each test consisted of eight clear plastic cups marked with a random three-digit code and containing approximately 50 mL of either one of the prepared solutions or distilled water. Panelists were asked to identify the taste as sweet, salty, sour, bitter or tasteless. Three taste samples were repeated in the sequence. A copy of the questionnaire is given in Figure 6.

3.2.3.2 Odour Recognition Panelists were also tested for the ability to correctly identify common odours. Odour screening tests were first conducted on July 22 and 24, 1986. The initial odour screening test consisted of solutions prepared in distilled water with the following added odours: iodine, bitumen, tuna fish packing water, almond extract, chlorine bleach, cloves, Ivory soap, onion, and vanilla extract. Although not a readily recognized odour, the bitumen was included to test recognition of petroleum related odours, and to familiarize the panelists with the type of odours that would be evaluated in later tests. Each test consisted of ten test tubes marked with random three-digit codes and containing approximately 100 mL of one of the solutions. Panelists were requested to identify each solution as closely as possible. One odour sample was repeated in the test sequence.

This test was repeated on November 13, 1986 for panelists who had missed the first test, and for the investigators who had prepared the initial solutions, and were thus biased in the knowledge of the odours. The second odour screening test consisted of solution prepared in distilled water with the following added odours: onion, Ivory soap, mouthwash, almond extract, chlorine bleach, cloves,

SCREENING TEST FOR TASTE

NAME _____ DATE_____

Eight coded samples are provided. Each of these cups contains weak water solutions of chemicals representing the four basic taste sensations. One or more of these may be a "blank" of distilled water, or may be a duplicate sample.

Rinse your mouth with the water provided and take a bite of cracker before tasting each sample. Taste each sample separately and in the order indicated. For each sample, record under "Taste Description" if the sample is tasteless, or has a sweet, salty, sour or bitter taste.

Sample Code Number	Taste Description
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Figure 6. Questionnaire for taste screening tests

curry, vinegar (acetic acid), alcohol (ethanol) and vanilla extract. No duplicate samples were given.

A copy of the questionnaire is given in Figure 7. Panelist responses were scored from 1 to 5, based on the closeness of the description to the actual odour.

3.2.3.3 Sequential Analysis Further screening used a Sequential Analysis procedure, as described by Amerine et al. (1965). Sequential Analysis tests were conducted on October 28 and 30, and November 4, 5 and 6, 1986. The procedure involves the specification of a reasonable ability acceptance criteria (p_1) and ability rejection criteria (p_0). Panelists are rated on the basis of the number of cumulative correct decisions made over a specified number of trials. Performance is related to two parallel straight lines of acceptance (L_1) or rejection (L_0). These are determined by the assigned values of p_0, p_1, ∂ and ß, where ∂ is the probability of rejecting an acceptable panelist (Type I error) and ß is the probability of selecting an unacceptable one (Type II error). The lines divide the plane into three regions: one of acceptance, one of rejection and one of indecision (requiring further testing).

The criteria used in this test were:

p_0 = maximum unacceptable ability = 0.70

p_1 = minimum acceptable ability = 0.45

∂ = ß = 0.05

The lines of acceptance and rejection (L_1 and L_0) are represented by the following equations:

$d_0 = a_0 + b_n$ (lower line L_0)

$d_0 = a_1 + b_n$ (upper line L_1)

SCREENING TEST FOR ODOUR

NAME _____ DATE _____

Ten coded samples are provided. Each of these test tubes contains a dilute solution of a compound having a typical odour

Sniff each sample separately and in the order indicated. Rinse your mouth with the water provided and take a bite of cracker after smelling each sample. Wait approximately 15 seconds between samples. Record your description of each sample under "Odour Description".

Sample Code Number	Odour Description
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Figure 7. Questionnaire for odour screening tests.

where: n = the total number of trials

d = the cumulative number of correct decisions

b = the slope of the lines

a = the intercept on the vertical axis

The slopes and intercepts are calculated as follows:

$$b = -k_2 / (k_1 - k_2)$$

$$a_0 = -e_1 / (k_1 - k_2)$$

$$a_1 = -e_2 / (k_1 - k_2)$$

where: $k_1 = \log p_1 - \log p_0$

$k_2 = \log (1 \quad \rho_1) - \log (1 - p_0) = \log q_1 - \log q_0$

$e_1 = \log \beta - \log (1 - \partial)$

$e_2 = \log (1 - \beta) - \log \partial$

The number of tests (n) required to satisfy the specified values of p_0, p_1, ∂ and β (i.e. the number of tests required until a panelist's cumulative responses fall into either the acceptance or rejection region) are calculated on the basis of special values of p, namely 0, p_0, p_1 and 1:

For $p = 0$ (no ability)

$$n_0 = e_1 / k_2$$

For $p = p_0$ (maximum unacceptable ability)

$$n_{P0} = [(1 - \partial) e_1 + \partial e_2] / (p_0 k_1 + q_0 k_2)$$

For $p_0 = p_0$ (minimum acceptable ability)

$$n_{P1} = [\beta e_1 + (1 - \beta) e_2] / (p_1 k_1 + q_1 k_2)$$

For $p_0 = 1$ (infallible ability)

$$n_1 = e_2 / k_1$$

For the stated criteria:

$k_1 = 0.1919$ $\quad k_2 = -0.2632$

$e_1 = -1.2788$ $\quad e_2 = 1.2788$

and

$n_0 = 5$ $\quad n_{P0} = 20$

$n_{P1} = 21$ $\quad n_2 = 7$

Therefore, approximately 21 tests were required. This number of tests ensured that the values of p_0 , p_1 , ∂ and ß as specified are satisfied.

The values of the slope and intercepts were:

$b = 0.578$ $\quad a_0 = -2.81$ $\quad a_1 = 2.81$

The equations for L_1 and L_0 were:

$L_0 : d_0 = -2.81 + 0.578\, n$

$L_1 : d_1 = 2.81 + 0.578\, n$

The Sequential Analysis test used was a paired difference test, consisting of 21 paired samples of four concentrations of naphthalene in walleye tissue. The naphthalene concentrations were: (1) 0 mg/kg (control); (2) 0.5 mg/kg; (3) 1.0 mg/kg; and (4) 2.0 mg/kg. These four concentrations were randomly paired, and presented to the panelists in three sessions of 7 pairs each. The questionnaire used in the screening, including the instructions given to the panelists, is given in Figure 8.

The sample preparation procedure was as described in Section 3.2.2, except that only 5 g aliquots were used for each sample. The mechanics of the sensory evaluation are described in Section 3.2.5. The samples were evaluated for odour only.

Sequential Analysis Set No. _____

SCREENING TEST FOR FISH TAINTING

NAME _____ DATE _____

Seven pairs of coded samples are provided. Each of these foil packets contains a sample of fish. Some samples have been artificially "tainted" with varying concentrations of a common household substance (moth balls). Other samples are untainted.

To evaluate the samples, tear off the end of the foil packet and open it as much as possible. Sniff each pair of samples separately and in the order indicated. Circle the sample within each pair which is most tainted. After you have completed your evaluation, close each packet by folding over the open end.

Rinse your mouth with the lemon water provided and take a bite of cracker after smelling each sample pair. Wait approximately 15 seconds between sample pairs.

Pair	Samples	
1	_____	_____
2	_____	_____
3	_____	_____
4	_____	_____
5	_____	_____
6	_____	_____
7	_____	_____

Figure 8. Questionnaire for Sequential Analysis screening tests.

The Sequential Analysis test served to evaluate both the ability of the panelists to discriminate differences in the specific attribute under study at the required level of sensitivity, and the ability to repeat this discrimination. It also provided basic training in the mechanics of the sensory evaluation procedures to be used and introduced the panelists to the medium used in the tests.

3.2.4 Sensory Panel Training

The selected panelists participated in three separate investigations on fish tainting related to oil sands wastewaters. Therefore, training in the mechanics of the sensory evaluation, including how to physically handle the samples and how to smell them for optimization of olfactory sensation, were provided in both the Sequential Analysis screening tests and in the paired difference tests conducted in the other two investigations.

Although the Consistent Series Threshold Method is a form of the paired difference test, additional training was provided specific to the threshold evaluation procedure. The panelists were trained using a minimum of one, and occasionally two, preliminary range determination tests for each compound to be evaluated. These tests served to train the panelists in the threshold evaluation procedure, to familiarize them with the specific compounds to be evaluated and to pinpoint the optimum range of concentrations for the actual threshold evaluation tests.

3.2.5 Sensory Threshold Evaluation Procedures

Twelve samples were presented to each panelist for each replication of the test. Six of the samples contained the different concentrations of the chemical compound to be evaluated. Three "blank" untainted samples were inserted

randomly amongst the tainted samples. The positions of the "blanks" in the series were different for each replication. The panelists were aware "blank" samples were present, but were not told the number of "blanks". To ensure that the spiked samples were always being compared to a reference of the same temperature, a reference "blank" sample was included for comparison with every set of three samples. The tainted samples were arranged in ascending order of concentration.

Sensory panel evaluations, including preliminary range determination tests, were conducted from November 25, 1986 to April 9, 1987. Panels were held on Monday, Tuesday and Thursday every week of this period, with the exception of a three week break over the Christmas holidays. All three replications for a compound were conducted within the week period. Amerine et al. (1965) cite literature suggesting that olfactory sensitivity decreases after a meal. Therefore, the sessions were conducted at 4:30 p.m., just before the evening meal, to maximize threshold sensitivity.

As in the preliminary tests, all panel sessions were held in a special sensory testing room designed to control background odours, room temperature, humidity and distracting activity that may interfere with the taste and odour evaluation. The room was equipped with individual booths and red lights to disguise possible color differences in the flesh. Figure 9 illustrates the layout of the booth for the sensory tests.

Each booth contained a hot plate. For presentation to the sensory panel, the coded samples, vacuum sealed with four packs to a bag, were further vacuum sealed in one polyethylene bag to prevent leakage. These bags were placed in 1 L beakers containing approximately 750 mL of hot water. These beakers were placed on the hot plates and heated to a temperature of 60°C. The temperature was

Figure 9. Sensory booth layout

increased from the 40°C used in preliminary tests on the basis of panelist preference. The samples remained sealed in the polyethylene bag from the time of preparation until opened by the panelist for evaluation. This ensured that there was no loss of volatiles prior to the sensory evaluation. Razor blades were provided to open the polyethylene bags. Panelists were requested to only have one packet open at one time to prevent cross-over of odours between samples.

Panelists were given a dilute lemon solution (approximately 1 tbsp. lemon juice to 2 L of room temperature water) as a rinsing agent, and unsalted soda crackers were provided to clear the palate between samples. Panelists were requested to evaluate if each sample was different from the reference. To minimize actual consumption of the chemical compounds, the panelists were instructed to taste the samples only if no odour was detected, as recommended by Shumway and Palensky (1973). Panelists were also told not to swallow any samples, but rather to expectorate the samples after the evaluation. The questionnaire and instructions provided to the panelists are given in Figure 10.

3.3 TISSUE ANALYSIS

The spiked fish tissue was analyzed to confirm the concentrations of the chemical in each sample, to ensure that the samples were homogeneously blended, and to compare known and extracted concentrations. Twenty gram samples of the minced, spiked fish tissue were extracted using a procedure involving drying, Soxhlet extraction, gel permeation chromatography (GPC) separation, and florisil clean-up.

CONSISTENT SERIES THRESHOLD DETERMINATION TEST

NAME _____ DATE _____

CHEMICAL COMPOUND _____

Nine coded samples are provided. Some of these samples are tainted and some are untainted "blanks". The tainted samples are arranged in order of increasing concentration. The coded samples are packaged in groups of three. An untainted reference sample marked "R" is provided for comparison in each packet of three samples.

To evaluate the samples, tear off the end of the foil packet and open it as much as possible. Sniff each coded sample separately and in the order indicated. Compare the sample with the reference and determine if there is a difference in odour. If a difference cannot be detected by smelling the samples, place a small portion of the sample in your mouth and determine if there is a difference in taste between the coded sample and the reference. Indicate if there is a difference from the reference by recording "yes" or "no" beside the sample code.

Rinse your mouth with the lemon water provided and take a bite of cracker after evaluating each sample. Wait approximately 15 seconds between samples.

Note: Only taste the sample if an odour difference cannot be detected. It is only necessary to introduce the sample to your mouth - you need not chew it. Do not swallow the sample. Expectorate the fish sample into the paper cup after completing your evaluation.

Sample Code	**Is there a difference from the reference? (indicate yes or no)**
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Figure 10. Questionnaire for the threshold evaluation tests.

3.3.1 Drying and Soxhlet Extraction

The 20 g sample was mixed with 80 g of anhydrous Na_2SO_4 to remove water bound within the tissue (Hesselberg and Johnson 1972). The blended tissue was extracted in a glass thimble packed with 20 mm of celite (to prevent the porous plate at the bottom of the thimble from being plugged with lipids), the blended tissue/Na_2SO_4 mixture, and a plug of glass wool. The celite and glass wool were previously extracted with dichloromethane (DCM). The prepared thimble was placed in a Soxhlet apparatus and refluxed for approximately 8 hours with 300 mL of DCM, as described by Archer and Crosby (1967). The extract with reduced in volume to approximately 8 mL using a Kuderna-Danish (K-D) apparatus held in a water bath maintained at 65°C. This extract contained lipid material and hydrocarbons present in the tissue.

3.3.2 GPC Separation

GPC separation was used to separate the lipids from the volatile hydrocarbon compounds in the Soxhlet extract. Separation is based primarily on differences in molecular size. The molecular weights of the petroleum compounds used in the threshold evaluations are between 106 and 184, while those of most lipids are between 600 and 1500. The procedure used was adapted from Stalling et al. (1972) and Vassilaros et al. (1982b).

Six glass chromatography columns of 600 mm by 19 mm (internal diameter) were prepared by placing a plug of DCM extracted glass wool at the bottom. Bio-Beads (S-X3) were soaked overnight in a 1:1 solvent mixture of DCM/hexane, then wet-packed to a depth of 500 mm in the column and topped with a 20 mm protective layer of sand. However, the sand layer became plugged with

lipid material and was later replaced with DCM extracted glass wool. These columns were used for all extractions, and were cleaned by running approximately 250 mL of DCM/hexane through the columns following each separation. Prior to use, an elution profile was determined for each column. These profiles showed the percent recovery of the desired compound in each successive 10 mL of the eluate.

The separation procedure involved the following steps:

1. The DCM/hexane solvent was drained just into the glass wool.
2. The concentrated 8 mL extract from the Soxhlet extraction was increased to 10 mL with DCM, added to the top of the GPC column and drained into the glass wool.
3. The K-D apparatus was rinsed with 10 mL DCM/hexane. The rinse was added to the top of the column, and again drained into the glass wool.
4. A reservoir containing 230 ml DCM/hexane was placed on top of the column, and allowed to drain freely.
5. Based on the elution profiles, the first 75 mL was collected and discarded.
6. The next 75 mL was collected, and reduced to 8 mL in a K-D apparatus held in a 80°C water bath.

3.3.3 Florisil Cleanup

Florisil was used to remove the remaining lipid material in a procedure described by Hesselberg and Johnson (1972). The florisil was activated by heating 10 g samples in 25 mL scintillation vials for 4 hours in a muffle furnace at 400°C. The samples were cooled in a dessicator, then sealed with aluminum lined plastic

caps and teflon tape. Because florisil separates compounds based on polarity, the use of a nonpolar eluant like hexane required that the florisil be partially deactivated to prevent adsorption of all organic compounds. The florisil was deactivated by the addition of either 5% or 10% deionized water (by volume) to each sample, depending on the pre-determined activation potential of each prepared batch.

Florisil columns were prepared by placing a DCM extracted glass wool plug at the bottom of a 400 mm by 10 mm (internal diameter) glass chromatography column. Ten grams of partially deactivated florisil was wet-packed in the column using hexane. A 20 mm layer of Na_2SO_4 was added to the top of the florisil to protect against water contamination. A separate florisil column was prepared for each extraction.

The cleanup procedure involved the following steps:

1. The hexane was drained just into the Na_2SO_4 layer.
2. The GPC extract was brought up to 10 mL with hexane, added to the top of the column and drained into the Na_2SO_4 layer.
3. The K-D apparatus was rinsed with 10 mL of hexane. The rinse was added to the top of the column and drained into the Na_2SO_4 layer.
4. An additional 40 mL of hexane was added to a 100 mL reservoir at the top of the column, and drained into the Na_2SO_4 layer.
5. The entire 60 mL of added extract and hexane were collected and reduced to 8 mL in a K-D apparatus held in a 80°C water bath. The 8 mL extract was further reduced to 1.0 mL under a stream of nitrogen in a N-EVAP.

3.3.4 GC Analysis

Acenaphthene was added as an internal standard to the final 1.0 mL fraction before it was analyzed by Gas Chromatography - Flame Ionization Detector (GC-FID).

A 2.0 µL aliquot of the extract was withdrawn using a Hamilton 10 µL syringe, and injected into a 30 m by 0.25 mm (internal diameter) fused silica capillary column with DB-5 coating (J & W Scientific).

Two flame ionization detectors were used for GC analysis. The first operated in a Varian Model 3500 capillary GC. The flow rate was 3 mL/min, and the split ratio was 50. The oven temperature was programmed for 40°C for 1 minute, an increase of 8°C per minute to a final column temperature of 300°C, and held at 300°C for 15 minutes. Injection port and detector temperatures were 270°C and 300°C, respectively. Peak areas were measured with a Varian Model DS604 data system.

The second FID was in a Hewlett-Packard Model HP 5880A series capillary GC. The over temperature program, and injection port and detector temperatures were the same as for the Varian 3500. Peak areas were measured with a HP 5880A computing integrator.

3.3.5 Quality Control and Percent Recoveries

Quality control involved the addition of 1.0 mL of a standard "cocktail" of all compounds to clean, control walleye tissue. The concentrations of each compound in the standard, summarized in Table 10, were similar to those used in the threshold determinations. The standard was added directly to the blended tissue/Na_2SO_4 mixture in the Soxhlet thimble and subjected to the extraction,

Table 10. The concentration of each compound in the standard "cocktail".

Compound	Concentration (ppm)
Naphthalene	5.08
1-Methylnaphthalene	4.072
2,6-Dimethylnaphthalene	20.16
2,3,5-Trimethylnaphthalene	20.375
Benzothiophene	4.022
Dibenzothiophene	4.128
p-Xylene	20.62
2,5-Dimethylphenol	5.00

cleanup and analysis procedure as described. As the purpose of the quality control standards was strictly to monitor the efficiency of the analytical procedure, these samples were not subjected to the blending process used in the actual samples. These quality control samples were also used to determine extraction recoveries for each compound in each of the six GPC columns.

All glassware used in the analysis was cleaned by soaking for a minimum of 6 hours in a Nalgene bath containing a laboratory glassware cleaning detergent, then rinsed with tap water and acetone, respectively, and baked at 325°C for 4 hours.

3.4 MATERIALS

The chemicals used in the spiking of the fish flesh, and in the preparation of the standard "cocktail" were all pesticide grade and obtained from Fisher Scientific Co.

The solvents used in the extraction and cleanup process were either pesticide grade or HPLC grade. The acetone, DCM and hexane were obtained interchangeably from Fisher Scientific Co., Caledon Laboratories Ltd. and Anachemica Chemicals Inc.

The florisil (60 - 100 mesh), celite (545) and Na_2SO_4 (anhydrous) were purchased from Fisher Scientific Co. The Bio-Beads S-X3 were obtained from Bio-Rad Laboratories Inc.

4. RESULTS AND DISCUSSION

4.1 SELECTION OF SENSORY THRESHOLD METHOD

The odour detection thresholds for each replication of the two testing methods are summarized in Table 11. Several general observations can be made from these values, the most significant of which is the variability of the results between the testing methods. In no replication of the experiment are the threshold values assigned by each panelist the same for each test, although common samples were used. In addition, the threshold values for the Consistent Series Test appear to be consistently lower than those for the Modified Triangle Test.

Threshold values also appear to vary considerably between replications of the tests. The fish used in Replication #2 was of questionable freshness, and the strong odour of the untainted fish may have biased the results for this replication. However, it is the values determined in Replication #3, where the fish was of comparable quality to that used in Replications #1 and #4, that exhibit the most discrepancy. The reason for this anomaly is not known.

In general, the threshold values decrease with subsequent replications of the experiment, suggesting that increased training of the panelists through repeated replications may increase their ability to detect the benzothiophene odour in the fish tissue.

The statistical significance of these observations is determined in the following analyses of the results.

Table 11. Summary of odour thresholds (mg/kg wet weight) of benzothiophene for each replication of the two testing methods.

Panelist	Replication #1		Replication #2		Replication #3		Replication #4	
	Consistent Series	Triangle Test	Consistent Series	Triangle Test	Consistent Series	Triangle Test	Consistent Series	Triangle Test
1	0.250	0.500	1.000	0.125	>1.000	>1.000	0.125	1.000
2	0.500	1.000	0.500	>1.000	>1.000	>1.000	0.250	0.500
3	0.063	>1.000	0.031	0.250	>1.000	1.000	0.031	0.125
4	0.500	>1.000	0.500	1.000	0.031	1.000	0.031	0.063
5	>1.000	>1.000	0.500	>1.000	1.000	>1.000	0.500	0.250

Note: A detection threshold of >1.000 indicates that the panelist could not detect the highest concentration of 1.000 mg/kg.

4.1.1 Odour Detection Threshold Values

Because of the small number of actual data points obtained, the results of each replication were pooled in the determination of odour threshold values. In accordance with the method described by A.S.T.M. (1968a), the results were summarized for each test, and the proportion of times each stimulus was reported was determined. The proportions were then graphed against the stimulus values (Figure 11).

The absolute or detection threshold is defined as the level of stimulus noticed 50% of the time. The detection thresholds for the Consistent Series Test and the Modified Triangle Test are 0.335 mg/kg and 0.800 mg/kg, respectively.

Detection threshold values are usually reported as a range rather than an absolute number to represent the natural variability of the human population in odour discrimination (Hamilton 1978). However, in many of the evaluations the panelists could not detect the highest concentration, indicating the sample concentrations were below the detection limits of the panelists. In addition, the selection of the lowest concentration in some evaluations could indicate that this concentration was above the detection limit of these panelists (Table 11). Therefore the range of concentrations for the determined threshold values are beyond the limits of the concentration range used in this experiment.

4.1.2 Comparison of Testing Methods

The t-test for paired data was used to evaluate the differences between the threshold values for each testing method. This test is applicable because each set of paired values were drawn from one panelist. In the case of paired variables, the difference between the two samples is considered the variate and is compared with

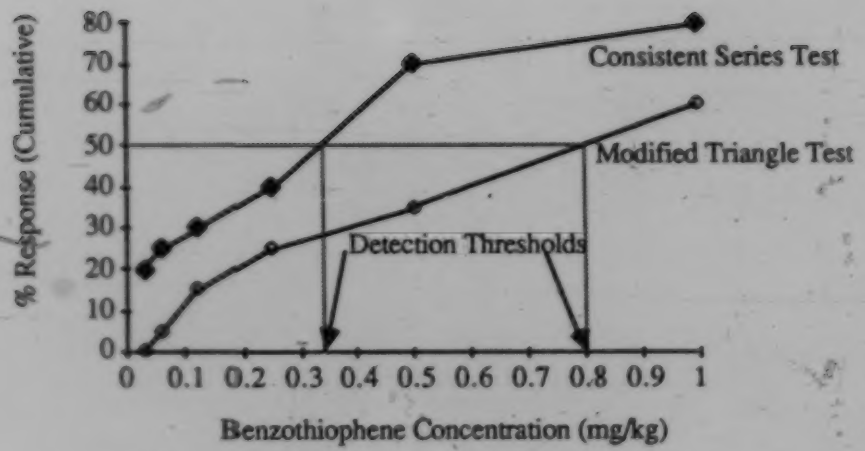


Figure 11. Odour detection thresholds for the Consistent Series and Modified Triangle tests.

the standard deviation of the mean differences. Although this eliminates the test-to-test variation, this advantage is offset by a loss in precision because the standard deviation is based on fewer degrees of freedom (Kennedy and Neville 1976).

For the purposes of these evaluations, threshold values that were above the concentration range presented (recorded in Table 11 as >1.000 mg/kg) were assumed to be at the next concentration interval, in this case 2.000 mg/kg. Table 12 summarizes the test statistics for the paired t-test evaluation of the difference between panelist thresholds for the Consistent Series and Modified Triangle tests. The threshold values are significantly different at the 0.05 confidence level. As the presentation order of the two tests was alternated between replications, the detrimental effect of sensory fatigue on the second test can be eliminated as a possible reason for the difference in threshold values. The threshold values determined by the Consistent Series Test are thus significantly lower that those obtained using the Modified Triangle Test. Bruvold (1977), in comparing analogous literature values for organic substances commonly found in water, also found that the Consistent Series Test consistently produced lower threshold values that did the Triangle Test. Rosen et al. (1962) reported that the Consistent Series method yielded an odour threshold concentration range for organic chemicals that overlapped that of the triangle procedure.

Sensory fatigue, caused by either overextending the sensory system with too many tests or by volatile chemicals that partially deaden olfactory response, has been shown to significantly affect threshold odour determinations (Hamilton 1978). The enhanced sensitivity of the Consistent Series Test is probably due to the fewer number of evaluations required, and hence the reduced opportunity for either physical or physiological sensory fatigue. This test also requires the preparation of

Table 12. Paired t-test values for comparisons between and within the Consistent Series and Modified Triangle tests.

Comparison	d.f.	Mean difference	Paired t-value	$t\sqrt{,.05}$[1]	Significant
Consistent Series & Modified Triangle Test - all values	19	0.450	2.569	2.093	Yes
Consistent Series Test Replications #1 & #4	4	0.475	1.783	2.776	No
Modified Triangle Test Replications #1 & #4	4	1.112	2.309	2.776	No
Consistent Series & Modified Triangle Tests - Repl. #4	4	0.200	1.070	2.776	No

1 Kennedy and Neville (1976)

fewer samples. The Consistent Series Test is therefore preferred for use in subsequent threshold determinations.

4.1.3 Evaluation of the Relative Ability of the Panelists

The Sequential Analysis procedure, described in Section 3.2.3.3, was used to assess the relative ability of each panelist in the odour determinations. For the purposes of this part of the study, a "correct" decision indicates that the panelist's threshold detection value for that replication was equal to or less than the overall detection threshold calculated on the basis of all panelists in all replications (0.335 mg/kg for the Consistent Series Test and 0.800 mg/kg for the Modified Triangle Test). A detection threshold below this value is deemed an "incorrect" decision. In this manner, the panelists are being evaluated against the cumulative response of the group.

Because the panelists used in this preliminary study were essentially untrained, the minimum acceptance criteria (p_0) was decreased from that used in the subsequent screening tests. Therefore, for this experiment, the assigned values were $p_0 = 0.40$, $p_1 = 0.70$, and $\partial = \beta = 0.05$. Using the series of computations described in Section 3.2.3.3, a minimum of sixteen tests would be required to fulfill these assigned specifications, although only four tests were performed for this experiment. The computed equations for the rejection and acceptance lines are:

$$L_0: \; d_0 = -2.35 + 0.553\, n$$

$$L_{01}: \; d_{01} = 2.35 + 0.553\, n$$

Figures 12 and 13 illustrate the sequential sampling patterns for the Consistent Series Test and Modified Triangle Test. Because the performance of all panelists falls within the region of indecision, more testing is required before

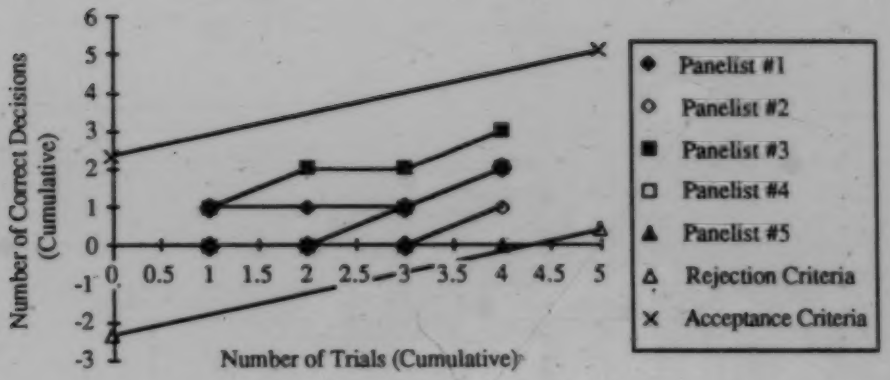


Figure 12. Sequential sampling pattern for the Consistent Series Test.

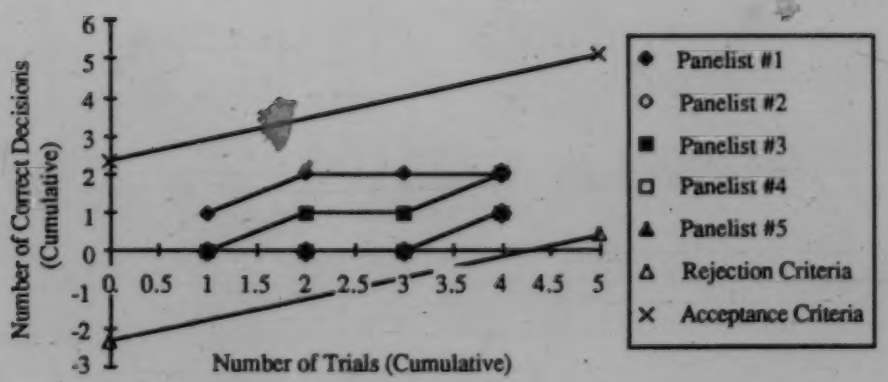


Figure 13. Sequential sampling pattern for the Modified Triangle Test.

decisions on acceptance or rejection can be made. However, the graphs do allow for relative observations on the ability of the individual panelists, and on the differences in performance between the two tests. The number of cumulative correct decisions is generally higher for all panelists in the Consistent Series Test than in the Modified Triangle Test. Panelist #3 is most proficient in the Consistent Series Test, followed by Panelists #1 and #4. In the Modified Triangle Test, the performance of Panelists #1 and #3 is the best. As the person originally specified as Panelist #1 was replaced after the second replication, the assessment of the relative performance of this panelist has little meaning. Overall, therefore, Panelist #3 exhibited the greatest ability and Panelist #5 the least ability in odour discrimination.

The relative performance of each panelist can also be assessed on the basis of the proportion of correct responses in the Modified Triangle Test that were accurately identified as being either "tainted" or "untainted" fish (Table 13). Although Panelist #3 correctly identified the odd sample more times than the other panelists, Panelist #4 had a higher proportion of responses that were accurately identified as to the nature of the sample. Overall, the performance of the five panelists was relatively similar in both the numbers and proportions of correctly identified responses.

4.1.4 Effect of Increased Training Through Replications

The observations of the investigator and the comments of the panelists indicated that the identification of the benzothiophene odour became easier with each subsequent replication of the experiment. To test this hypothesis, the detection thresholds at Replications #1 and #4 were determined and compared for each test.

Table 13. Proportion of correct responses in the Modified Triangle Test that were accurately identified as being either "tainted" or "untainted" fish.

Panelist	Replication #1		Replication #2		Replication #3		Replication #4		TOTAL	
	Correct Responses	% Accurately Identified	Correct Responses	% Accurately Identified	Correct Responses	% Accurately Identified	Correct Responses	% Accurately Identified	Correct Responses	% Accurately Identified
1	2	100	5	40	1	100	2	50	10	60
2	3	33	1	0	1	100	3	100	8	68
3	2	0	3	33	4	75	4	100	13	62
4	0	0	4	100	2	100	5	100	11	100
5	1	0	2	50	3	67	3	100	9	67
TOTAL	8	38	15	53	11	82	17	94	**51**	**71**

Figure 14 compares the threshold values for the two replications of the Consistent Series Test. The detection threshold value is again determined by the concentration noticed 50% of the time. The threshold value for Replication #1 is 0.32 mg/kg, and for Replication #4 is 0.09 mg/kg.

The comparison of threshold values for the Modified Triangle Test is illustrated in Figure 15. The data from Replication #1 were extrapolated to determine the approximate concentration corresponding to 50% of the responses. The detection thresholds for Replications #1 and #4 are, respectively, 1.24 and 0.19 mg/kg.

The differences between the individual panelist threshold values for the two replications of each test were compared using the paired t-test (Table 12). Although neither comparison indicates a significant difference, the mean differences between the replications are fairly high, at 0.475 and 1.112 for the Consistent Series and Modified Triangle tests, respectively.

The large difference between the thresholds for the Modified Triangle Test indicates that the effect of training is greater for this test. The more complicated nature of this test probably requires additional training to attain comparable proficiency to that achieved for the Consistent Series Test. This led to the speculation that the observed differences in the overall results of the tests might be less pronounced if the threshold values for Replication #4 only were compared. The comparison is illustrated in Figure 16. The threshold values are 0.09 and 0.19 mg/kg, respectively, for the Consistent Series Test and the Modified Triangle Test. These individual threshold values for this replication are not significantly different at the 0.05 significance level using the paired t-test (Table 12), indicating that

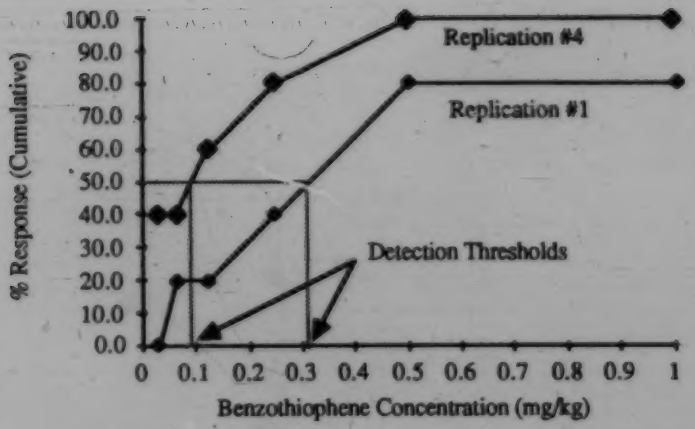


Figure 14. Comparison of detection threshold values at Replications #1 & 4 for the Consistent Series Test.

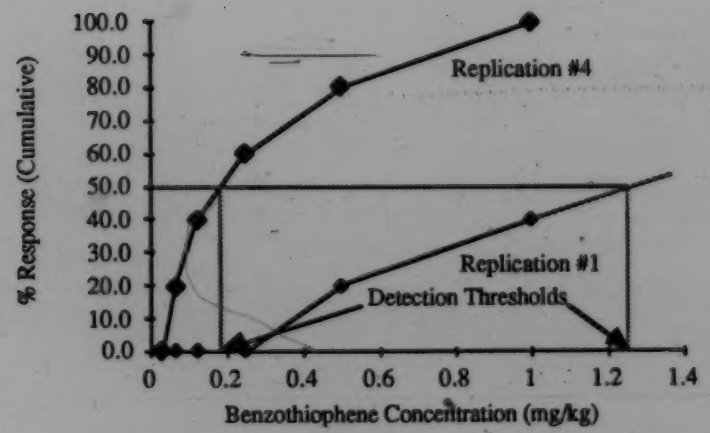


Figure 15. Comparison of detection threshold values at Replications #1 & 4 for the Modified Triangle Test.

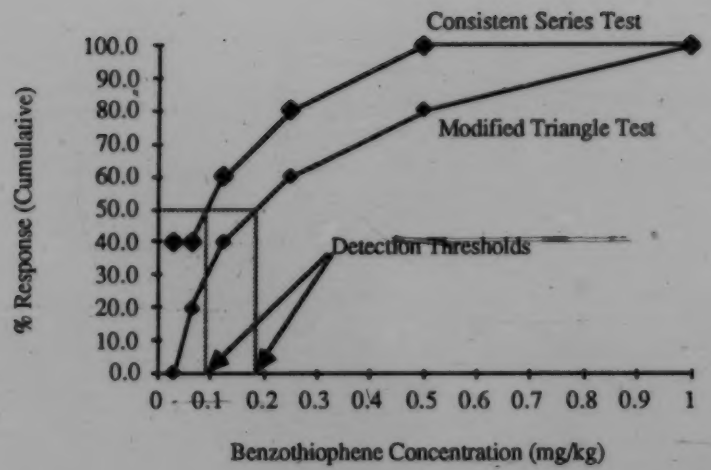


Figure 16. Comparison of detection threshold values at Replication #4 for the Consistent Series and the Modified Triangle tests.

further replications, and/or increased training of the panelists, might eliminate any overall difference in sensitivity between the two tests.

The effect of training through subsequent replications is also evident in the proportion of correct responses in the Modified Triangle Test that were accurately identified as being either "tainted" or "untainted" fish (Table 13). The proportion of accurately identified responses increased steadily from 38% in Replication #1 to 94% in Replication #4.

The relative performance of the panelists, and the enhanced odour discrimination achieved through subsequent replications, illustrates the necessity of pre-screening and intensive training of panelists in threshold determination tests. Foster (1968) and Wittes and Turk (1968) found that failure to observe these procedures is the major source of variability in the subjective measurement of odours.

4.2 SENSORY PANEL SCREENING AND SELECTION

4.2.1 Taste Recognition

Each panelist was scored by awarding a value of 1 for a correct response, and a value of 0 for an incorrect response. A summary of the taste scores for each panelist, and the cumulative scores for each sample is given in Table 14. Panelist scores ranged from a minimum of 4 (50% correct) to 8 (100% correct). Overall, the taste scores were high, with 67.9% of the panelists scoring the maximum of 8, 21.4% scoring 7, and 3.6% scoring 6, 5 and 4, respectively. The poor score exhibited by Panelist #24 was due to confusion between the terms "sour" and "bitter", and does not indicate ageusia.

Table 14. Taste recognition screening test results (1 = correct response and 0 = incorrect response.

Panelist No.	Taste Score								Total
	Sour	Blank	Salty	Bitter	Sweet	Bitter	Blank	Sour	
1	1	1	1	1	1	1	1	1	8
2	1	1	1	1	1	1	1	1	8
3	1	1	1	1	1	1	1	1	8
4	1	1	1	1	1	1	1	1	8
5	1	1	1	1	1	1	0	1	7
6	1	1	1	1	1	1	1	1	8
7	1	1	1	1	1	1	0	1	7
8	1	1	1	1	1	0	1	1	7
9	1	1	1	1	1	1	1	1	8
10	1	1	1	1	1	1	1	1	8
11	1	1	1	1	1	1	1	1	8
12	1	1	1	1	1	1	1	1	8
13	1	1	1	1	1	1	1	1	8
14	1	0	1	1	1	1	1	1	7
15	1	1	1	1	1	1	1	1	8
16	1	1	1	1	1	1	1	1	8
17	1	1	1	1	1	1	1	1	8
18	0	1	1	1	1	1	1	1	7
19	1	1	1	1	1	1	1	1	8
20	0	1	1	1	1	0	0	1	5
21	1	1	1	1	1	1	1	1	8
22	1	1	1	1	1	1	1	1	8
23	1	1	1	1	1	1	1	1	8
24	0	1	1	0	1	0	1	0	4
25	0	1	1	1	1	1	1	1	7
26	1	1	1	1	1	1	1	1	8
27	1	1	1	1	1	1	1	1	8
28	1	1	1	0	1	0	1	1	6
Total	24	27	28	26	28	24	25	27	209
% Correct	86%	96%	100%	93%	100%	86%	89%	96%	

The overall percent of each sample judged correctly shows that the panelists had no difficulty in recognizing salty and sweet tastes. More difficulty was encountered with the sour and bitter tastes, possibly due to an incorrect interpretation of these terms. Surprisingly, some difficulty was also encountered with the "blank" water samples. This could be the result of using distilled water, which often has a slightly bitter taste. Tap water was found to be better for sensory evaluation purposes, if it does not have any unusual taste or odour. Based on these results, tap water was used for rinsing purposes in the subsequent screening, training and evaluation tests.

4.2.2 Odour Recognition

As the description of odours is more subjective than the recognition of taste, a score of 0 to 5 was awarded based on the relationship of the description to the actual odour. The scoring procedure was adapted from that suggested by Vaisey Genser (1977). Five was the maximum score, and was given only if the description was completely accurate. A score of 4 was awarded if the description was synonymous with, but not identical to, the odour stimulus. A score of 3 indicated that the description was somewhat correct (within the same narrow class of odours as the actual odour), whereas a score of 2 indicated the description was somewhat incorrect (within the same broad class of odours as the actual odour). A score of 1 was awarded if the description was nearly incorrect, but still had some slight relationship to the odour stimulus. An absolutely incorrect response was given a score of 0. These scores were assigned by a single individual to ensure consistency of the evaluations.

A summary of the odour recognition scores is given in Table 15. As described in Section 3.2.3.2, two separate odour screening tests were conducted. Each panelist completed only one of the tests. The two tests differed somewhat in both the nature of the odour solutions and the order of presentation . The upper set of odour descriptions in Table 15 are those used in the initial odour screening test. The descriptions in parentheses are the odours used in the second screening test. The best score was attained by Panelist #28, who scored the maximum on all odour descriptions for a total score of 50. Panelist #7 showed the poorest score, accumulating only 20 points out of the possible 50. Panelist #11 was unavailable to complete this and subsequent screening tests.

Cloves were the most readily recognized odour. In a 1986 survey of 26,200 people across the United States, Gilbert and Wysocki (1987) found that of six representative odour samples, eugenol (cloves) scored highest among both sexes in identification.

4.2.3 Sequential Analysis

The panelists were scored in the Sequential Analysis test by awarding a value of 1 for correctly identifying the sample with the highest concentration of naphthalene in the paired comparison test, and a value of 0 for an incorrect evaluation.

A summary of the Sequential Analysis test results is given in Table 16. The numbers beneath each pair number represent the concentrations of naphthalene in mg/kg of the two paired samples. Panelists # 2, 3 and 27 scored the maximum of 21. The lowest score was attained by Panelist #9, who scored only 10. In general, the pairs of samples with the largest range in concentration were most frequently

Table 15. Odour recognition screening test results (Scoring based on: 5 = Correct; 4 = Nearly correct; 3 = Somewhat correct ; 2 = Somewhat incorrect; 1 = Nearly incorrect; and 0 = Incorrect).

Panelist No.	Odour Score										Total
	Iodine (Onion)	Bitumen (Soap)	Tuna Fish (Mouthwash)	Almond (Almond)	Bleach (Bleach)	Cloves (Cloves)	Soap (Curry)	Onion (Vinegar)	Vanilla (Ethanol)	Soap (Vanilla)	
1	3	3	5	5	1	5	5	3	5	5	40
2	0	3	5	5	5	5	3	5	4	1	36
3	2	3	5	3	0	4	5	5	3	5	35
4	3	5	0	5	3	3	5	5	5	5	39
5	0	1	5	5	3	5	5	5	5	5	39
6	0	5	5	5	5	3	5	5	3	5	41
7	3	2	0	3	0	2	5	2	3	0	20
8	2	2	5	5	0	5	5	5	3	0	32
9	0	3	3	1	5	5	5	1	5	5	33
10	5	5	5	5	5	5	5	5	4	5	49
11	-	-	-	-	-	-	-	-	-	-	-
12	2	5	5	5	5	5	4	5	1	4	41
13	3	4	5	5	3	3	5	5	5	5	43
14	1	5	5	2	5	3	5	2	3	5	36
15	5	5	5	3	5	5	2	5	5	5	45
16	3	5	5	5	0	5	5	5	5	5	43
17	2	5	2	5	5	5	4	5	5	5	43
18	4	5	1	1	5	3	0	5	5	0	29
19	3	2	5	5	0	5	0	0	5	0	25
20	3	5	5	4	3	5	5	5	2	5	42
21	5	5	5	5	5	5	3	5	5	5	48
22	4	2	4	5	5	5	5	5	5	2	42
23	2	3	5	4	3	3	5	5	4	5	39
24	3	4	5	5	5	3	5	5	4	5	44
25	5	5	5	5	5	5	5	5	3	5	48
26	5	5	5	5	3	5	5	5	5	5	48
27	3	5	5	5	5	5	5	3	5	5	46
28	5	5	5	5	5	5	5	5	5	5	50
Total	76	107	115	116	94	117	116	116	112	107	1076
Average	2.81	3.96	4.26	4.30	3.48	4.33	4.30	4.30	4.15	3.96	39.85

Table 16. Sequential Analysis screening test results (1 = correct response and 0 = incorrect response).

Panelist No.	Sequential Analysis Scores (Session #1)						
	Pair #1 (4 & 2)[1]	Pair #2 (2 & 8)	Pair #3 (8 & 0)	Pair #4 (8 & 4)	Pair #5 (8 & 2)	Pair #6 (2 & 8)	Pair #7 (0 & 4)
1	0	1	1	1	1	1	1
2	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1
4	1	1	1	0	1	1	1
5	-	-	-	-	-	-	-
6	1	1	1	1	0	1	0
7	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
9	1	0	0	0	1	1	1
10	0	1	1	0	1	1	0
11	-	-	-	-	-	-	-
12	1	1	1	1	1	1	1
13	1	0	1	1	1	1	1
14	0	0	1	1	1	1	1
15	1	0	1	1	0	1	1
16	0	1	1	0	1	1	1
17	1	1	1	1	1	1	1
18	1	1	1	1	0	1	1
19	1	1	0	1	1	1	1
20	1	1	1	1	1	1	0
21	0	1	1	1	0	1	1
22	1	1	1	1	1	1	1
23	-	-	-	-	-	-	-
24	0	1	1	1	1	1	1
25	1	1	1	1	1	1	1
26	1	1	1	1	1	1	0
27	1	1	1	1	1	1	1
28	1	1	1	1	1	1	1
Total	17	19	21	19	19	23	19
% Correct	74	83	91	83	83	100	83

[1] Bracketed numbers are the concentrations of naphthalene (mg/kg) in the two paired samples

continued . . .

Table 16. Continued.

Panelist No.	Sequential Analysis Scores (Session #2)						
	Pair #8 (0& 2)	Pair #9 (8 & 4)	Pair #10 (8 & 0)	Pair #11 (2 & 4)	Pair #12 (8 & 2)	Pair #13 (0 & 4)	Pair #14 (0 & 4)
1	0	1	1	1	1	0	1
2	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1
4	0	1	1	1	1	1	1
5	-	-	-	-	-	-	-
6	1	1	1	0	1	0	1
7	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-
9	0	0	1	0	0	1	1
10	1	1	1	1	1	1	1
11	-	-	-	-	-	-	-
12	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1
14	1	0	1	0	1	1	0
15	1	0	1	1	1	1	1
16	1	1	1	0	1	1	1
17	0	0	1	1	1	1	1
18	0	0	1	1	1	0	1
19	1	1	1	1	1	1	1
20	1	1	1	0	1	0	1
21	0	1	1	1	1	1	1
22	1	1	1	1	1	1	1
23	-	-	-	-	-	-	-
24	0	1	1	1	1	0	1
25	1	1	1	0	1	0	1
26	1	1	1	0	1	1	1
27	1	1	1	1	1	1	1
28	0	1	1	0	1	1	1
Total	15	18	23	15	22	17	22
% Correct	65	78	100	65	96	74	96

continued . . .

Table 16. Concluded.

Panelist No.	Sequential Analysis Scores (Session #3)							
	Pair #15 (4 & 8)	Pair #16 (8 & 0)	Pair #17 (0 & 4)	Pair #18 (2 & 0)	Pair #19 (2 & 0)	Pair #20 (8 & 0)	Pair #21 (2 & 4)	Total
1	0	1	1	0	0	1	1	15
2	1	1	1	1	1	1	1	21
3	1	1	1	1	1	1	1	21
4	0	1	1	1	1	1	0	17
5	-	-	-	-	-	-	-	-
6	1	1	1	1	1	1	1	17
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	0	0	0	1	1	1	0	10
10	1	1	1	0	0	1	1	16
11	-	-	-	-	-	-	-	-
12	1	1	1	0	1	1	1	20
13	1	1	1	1	1	1	1	20
14	1	0	1	1	1	1	1	15
15	1	1	1	0	1	0	1	16
16	1	1	1	1	0	1	1	17
17	1	1	1	1	1	1	1	19
18	1	1	1	1	0	1	1	16
19	1	1	1	1	1	1	1	20
20	1	1	1	0	1	1	1	17
21	1	1	1	1	1	1	1	18
22	1	1	1	0	1	1	1	20
23	-	-	-	-	-	-	-	-
24	1	1	1	0	1	1	1	17
25	1	1	1	0	1	1	1	18
26	1	1	1	0	0	1	0	16
27	1	1	1	1	1	1	1	21
28	1	1	0	1	1	1	1	18
Total	20	21	21	14	18	22	20	405
% Correct	87	91	91	61	78	96	87	84

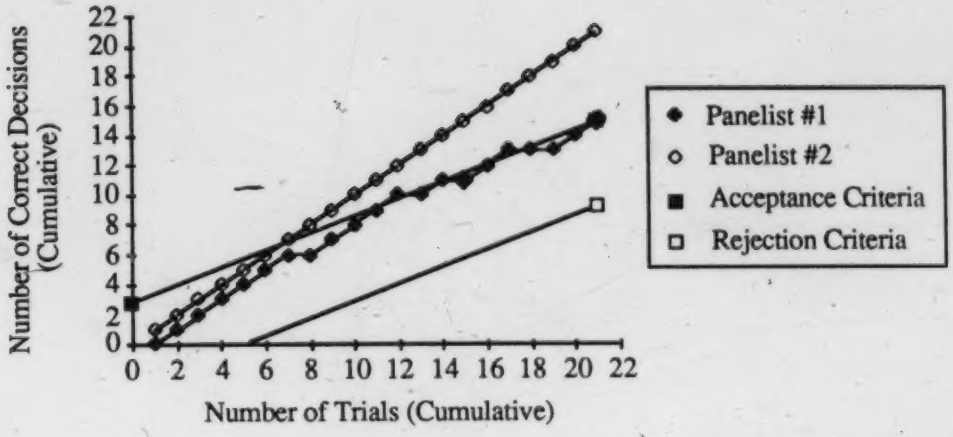
judged correctly. Panelists #5, 7, 8 and 23 were unavailable to complete this screening test.

In accordance with the prescribed evaluation procedures for this test, the cumulative correct scores for each panelist were plotted against the cumulative number of trials. These graphs are given in Figures 17 through 22. The performance of most panelists falls within the acceptance region, as defined by the assigned specifications. This indicates that they correctly identified more than 70% of the paired samples. The cumulative scores of panelists #1, 9 and 14 fall within the region of indecision, indicating that further tests would be required to determine if these panelists could meet the acceptance criteria. All panelists correctly identified more than 45% of the samples correctly, and could not be unequivocally rejected based on the ability rejection criteria.

4.2.4 Panelist Selection

A summary of the scores and the percent correct for each panelist is presented in Table 17 for the three screening tests. Only those panelists completing all of the screening tests were included in the selection process. The scores of these panelists were ranked, with 1 indicating the best score. Tie scores were awarded the same rank.

Unfortunately, it was not possible to select the ten top ranking panelists for the final panel because of availability and motivation. Panelists # 13, 15, 17 and 19 (ranking 2nd, 8th, 5th and 8th, respectively) were leaving the country and would not be available for the duration of the evaluation tests. Panelists #10 and 12 (ranking 6th and 3rd, respectively) were unwilling to continue with sensory testing involving tasting of the fish samples. Panelist #25 (ranking 6th) was one of the


Number of Correct Decisions (Cumulative)
0 2 4 6 8 10 12 14 16 18 20 22
Number of Trials (Cumulative)
Panelist #1
Panelist #2
Acceptance Criteria
Rejection Criteria

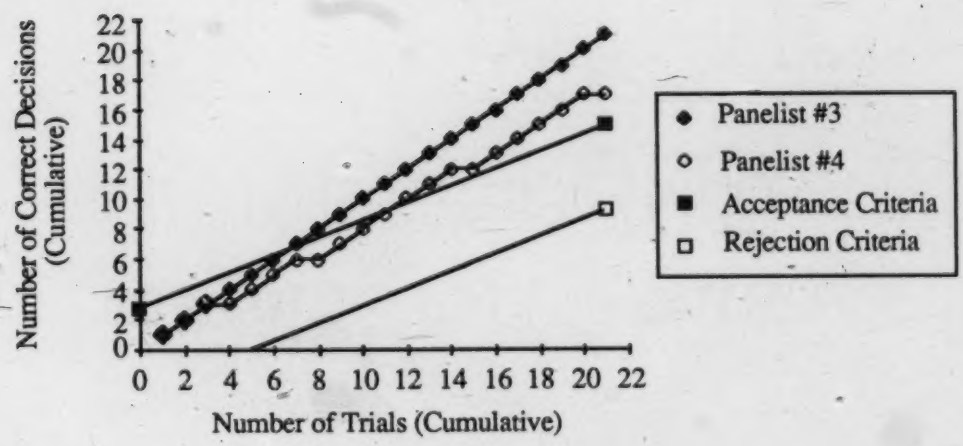
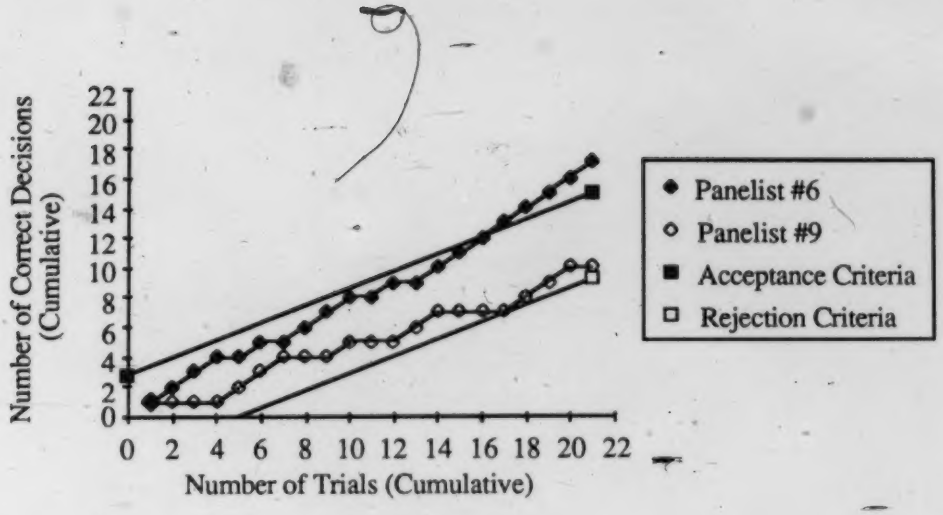

Number of Correct Decisions (Cumulative)
0 2 4 6 8 10 12 14 16 18 20 22
Number of Trials (Cumulative)
Panelist #3
Panelist #4
Acceptance Criteria
Rejection Criteria

Figure 17. Sequential Analysis results for Panelists #1, 2, 3 and 4.



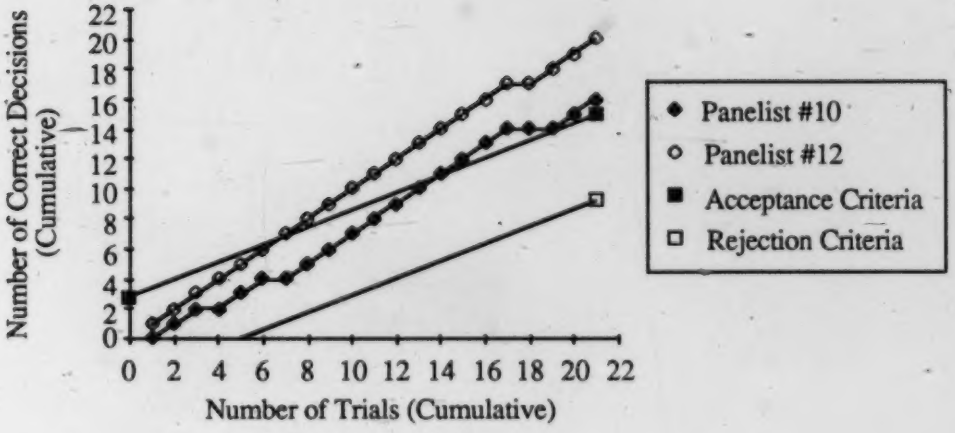
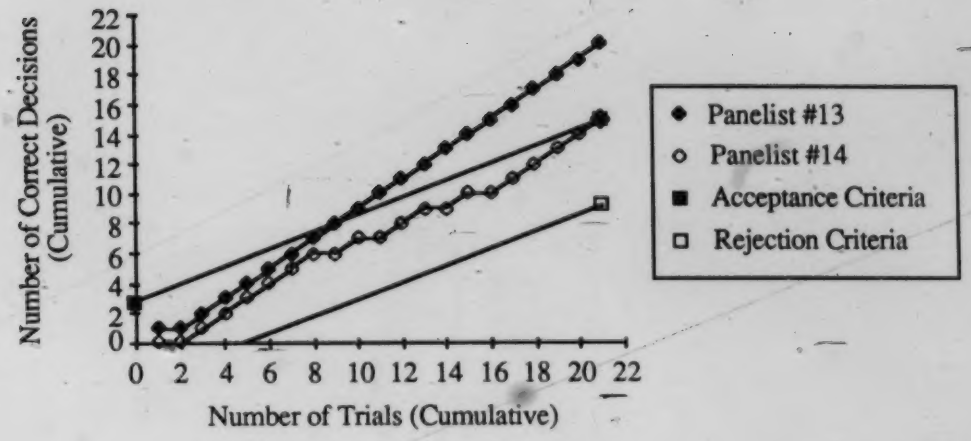


Figure 18. Sequential Analysis results for Panelists #6, 9, 10 and 12.



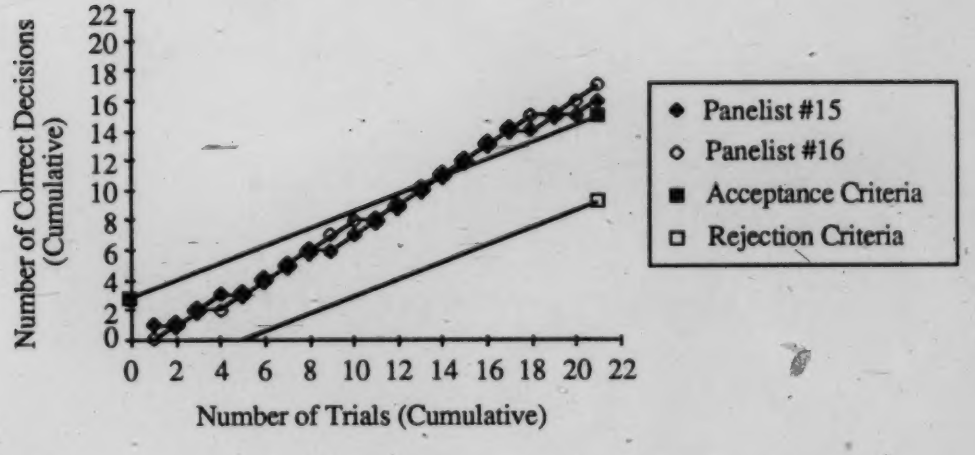
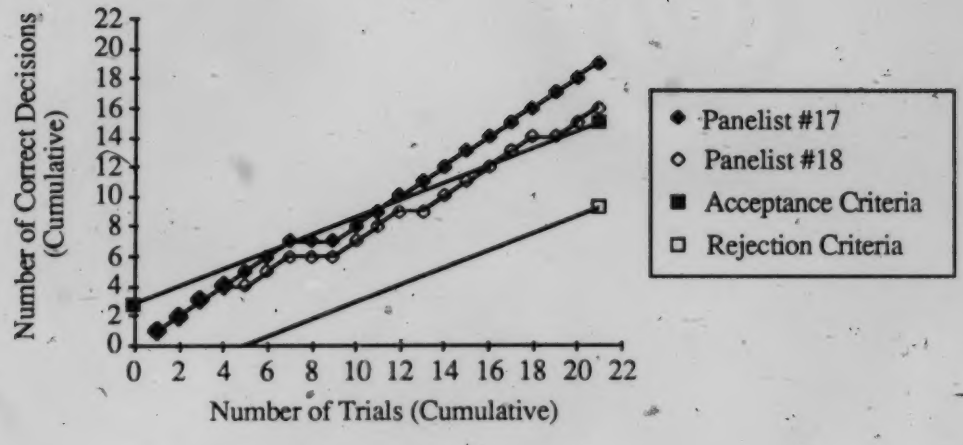


Figure 19. Sequential Analysis results for Panelists #13, 14, 15 and 16.


Number of Correct Decisions (Cumulative)
0 2 4 6 8 10 12 14 16 18 20 22
Number of Trials (Cumulative)
Panelist #17
Panelist #18
Acceptance Criteria
Rejection Criteria

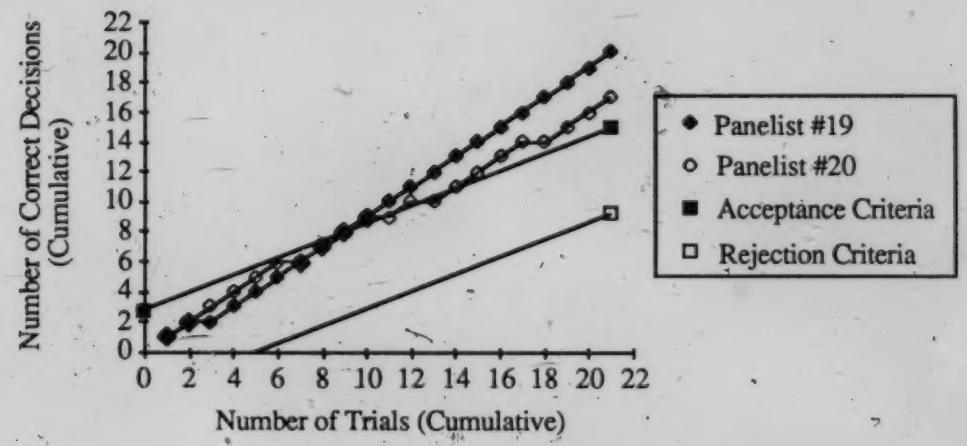

Number of Correct Decisions (Cumulative)
0 2 4 6 8 10 12 14 16 18 20 22
Number of Trials (Cumulative)
Panelist #19
Panelist #20
Acceptance Criteria
Rejection Criteria

Figure 20. Sequential Analysis results for Panelists #17, 18, 19 and 20.

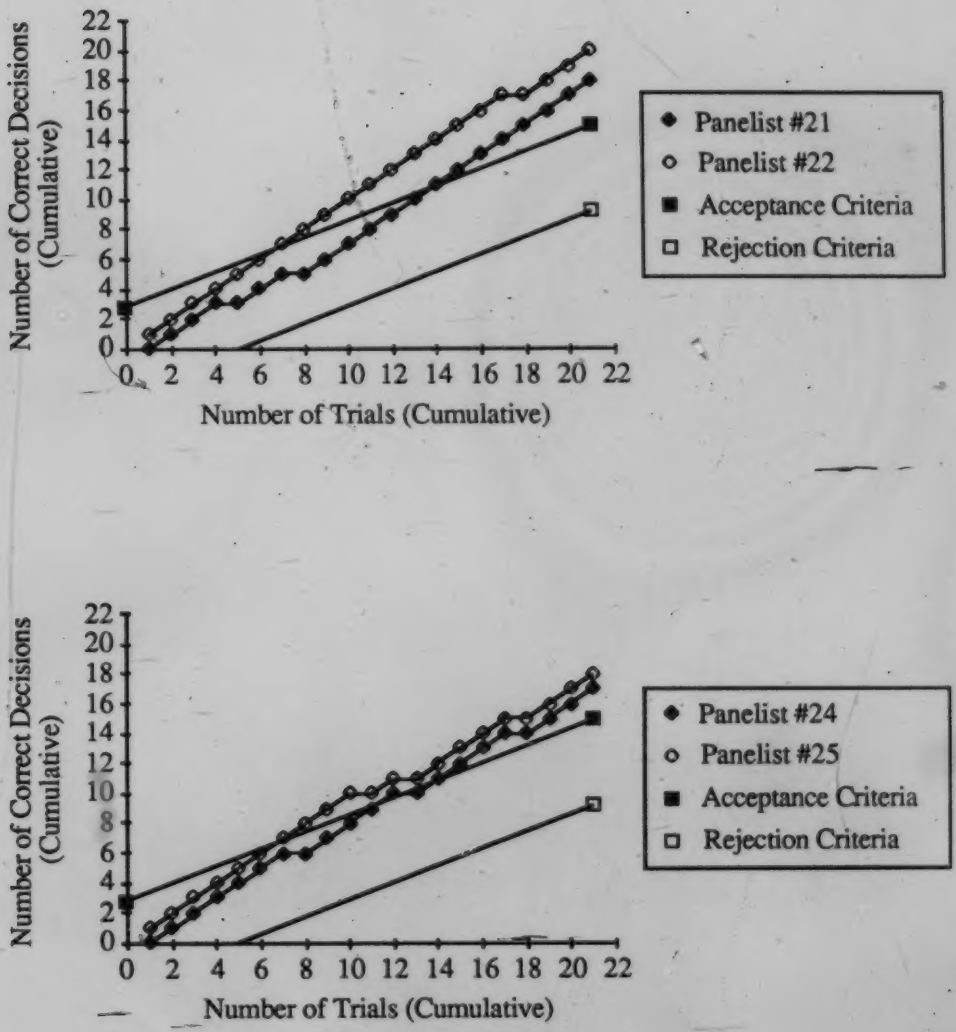
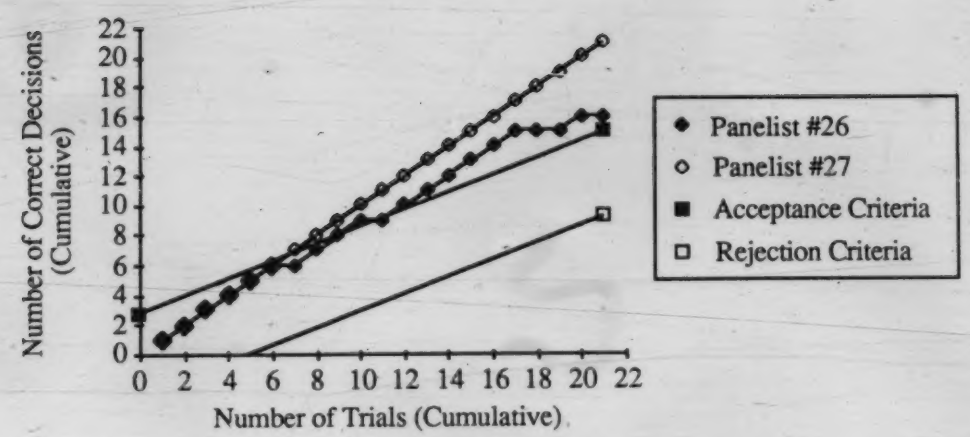


Figure 21. Sequential Analysis results for Panelists #21, 22, 24 and 25.


Number of Correct Decisions (Cumulative)
0 2 4 6 8 10 12 14 16 18 20 22
Number of Trials (Cumulative)
Panelist #26
Panelist #27
Acceptance Criteria
Rejection Criteria

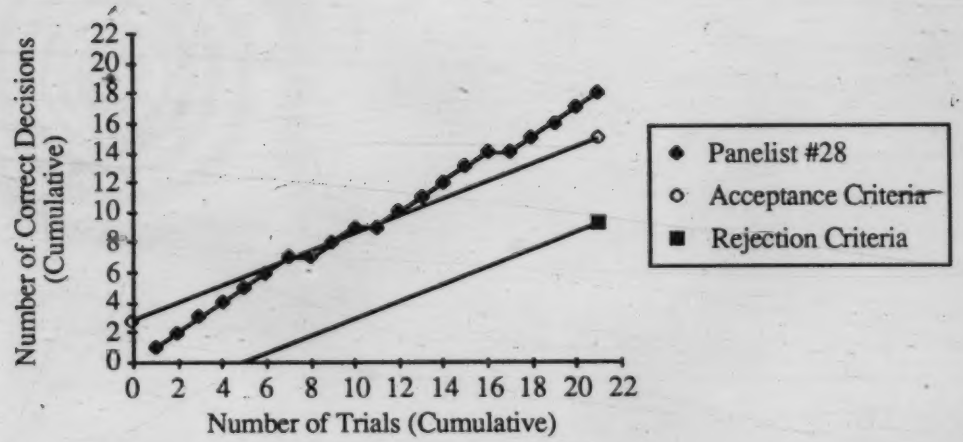

Number of Correct Decisions (Cumulative)
0 2 4 6 8 10 12 14 16 18 20 22
Number of Trials (Cumulative)
Panelist #28
Acceptance Criteria
Rejection Criteria

Figure 22. Sequential Analysis results for Panelists #26, 27 and 28.

Table 17. Screening results summary and panelist ranking.

Panelist No.	Screening Total	% Correct	Ranking
1	31	79%	10
2	36.2	93%	3
3	36	92%	4
4	32.8	84%	9
5	-	-	-
6	33.2	85%	8
7	-	-	-
8	-	-	-
9	24.6	63%	15
10	33.8	87%	6
11	-	-	-
12	36.2	93%	3
13	36.6	94%	2
14	29.2	75%	13
15	33	85%	8
16	33.6	86%	7
17	35.6	91%	5
18	28.8	74%	14
19	33	85%	8
20	30.4	78%	11
21	35.6	91%	5
22	36.4	93%	3
23	-	-	-
24	29.8	76%	12
25	34.6	89%	6
26	33.6	86%	7
27	38.2	98%	1
28	34	87%	6
Average	33.3	85%	

investigators involved in concurrent fish tainting studies and was not available for regular panel sessions. Therefore, the initial final panel selected consisted of Panelists #1, 2, 3, 4, 6, 16, 20, 21, 22 and 27. Panelists # 21 and 22 were unable to continue after completing four preliminary tests, and were replaced with Panelists #18 and 28. Panelist #26 was the investigator for this study, and participated in all tests. The integrity of the evaluations by the investigator were maintained on the basis that an assistant packaged the coded samples, and the evaluation was completed in advance of scoring the other panelists evaluations. Therefore, a final panel of eleven individuals was used.

The final panel was composed of five males and six females, ranging in age from 24 to 40 years. All panelists were non-smokers. The panelists came from a range of backgrounds relative to this experiment: one panelist was a chemist, and was familiar with the odours of many of the compounds used; two panelists were graduate students working in sensory evaluation with the Department of Foods and Nutrition; three panelists worked with fish with the Department of Zoology; one panelist was familiar with sensory evaluation procedures through work with the Department of Food Science; and two panelists were directly involved with this project.

For ease of future reference the final selected panelists were renumbered consecutively from 1 to 11 in subsequent testing.

4.3 THRESHOLD DETECTION VALUES

All compounds were screened in a single sensory panel session at an expanded range of concentrations. Because of relatively poor detectability by the panel, thiophene, 2-methylthiophene, toluene and mesitylene were not pursued for

detection threshold evaluation. The test concentration ranges and sensory results for these compounds are given in Table 18.

The concentration ranges used for the threshold detection evaluation of the remaining compounds were based on the results of the preliminary range determination tests. The concentration ranges used for the preliminary tests (Table 19) were derived from the available threshold data for the compounds in fish flesh and water (see Section 2.3). Although each successive concentration was based on a factor of two increase, some of the intervals were omitted to obtain the expanded range necessary to determine the appropriate threshold range for the panelists. Table 19 also shows the final concentration ranges used in the threshold evaluations as determined from the preliminary testing.

The individual panelist threshold values obtained for each replication are summarized for the selected compounds in Appendix B. The results of each replication of the threshold evaluations for these compounds were pooled to determine the overall threshold values. Individual tests were discounted if the panelist complained of an abnormality, such as an off-flavour reference sample, and if this test threshold was inconsistent with those of the other two replications.

The detection thresholds for each compound were determined using the A.S.T.M. (1968a) method described in Section 4.1.1. Figures 23 through 31 illustrate the derivation of the detection thresholds for each compound.

A survey of the panelists throughout the tests and following the completion of all evaluations indicated that eight of the eleven panelists attributed their assessment of the presence of a "tainting" compound primarily to the odour component. These panelists agreed that, in general, tasting the samples produced no additional organoleptic sensation. Those panelists that did experience some taste

Table 18. Test concentration range and sensory evaluation results for compounds not pursued for detection threshold evaluation.

1. Thiophene

Test concentration range: 0.031 to 4.00 mg/kg

Results: 8 panelists (73%) could not detect compound at 4.00 mg/kg
2 panelists (18%) detected compound at 4.00 mg/kg
1 panelist (9%) detected compound at 0.50 mg/kg

2. 2-Methylthiophene

Test concentration range: 0.031 to 4.00 mg/kg

Results: 3 panelists (27%) could not detect compound at 4.00 mg/kg
4 panelists (36%) detected compound at 4.00 mg/kg
2 panelists (18%) detected compound at 2.00 mg/kg
1 panelist (9%) detected compound 0.50 mg/kg
1 panelist (9%) detected compound at ≤ 0.031 mg/kg

3. Toluene

Test concentration range: 0.063 to 16.00 mg/kg

Results: 4 panelists (36%) could not detect compound at 16.00 mg/kg
5 panelists (45%) detected compound at 16.00 mg/kg
1 panelist (9%) detected compound at 8.00 mg/kg
1 panelist (9%) detected compound at 4.00 mg/kg

4. Mesitylene

Test concentration range: 0.063 to 16.00 mg/kg

Results: 5 panelists (45%) could not detect compound at 16.00 mg/kg
4 panelists (36%) detected compound at 16.00 mg/kg
2 panelists (18%) detected compound at 8.00 mg/kg

Table 19. The preliminary and final threshold concentration ranges used for each compound pursued for threshold evaluation.

Compound	Preliminary Concentration Range (mg/kg)	Final Concentration Range (mg/kg)
Naphthalene	0.031 to 4.000	0.063 to 2.000
1-Methylnaphthalene	0.031 to 4.000	0.125 to 4.000
2,6-Dimethylnaphthalene	0.062 to 8.000	2.0 to 64.0
	0.50 to 32.00	
2,3,5-Trimethylnaphthalene	0.50 to 32.00	2.0 to 64.0
Benzothiophene (Test #1)	0.031 to 4.000	0.063 to 2.000
Benzothiophene (Test #2)		0.0063 to 0.2000
Dibenzothiophene	0.031 to 4.000	0.25 to 8.00
p-Xylene	0.063 to 16.000	1.0 to 32.0
2,5-Dimethylphenol	0.063 to 16.000	0.016 to 0.500

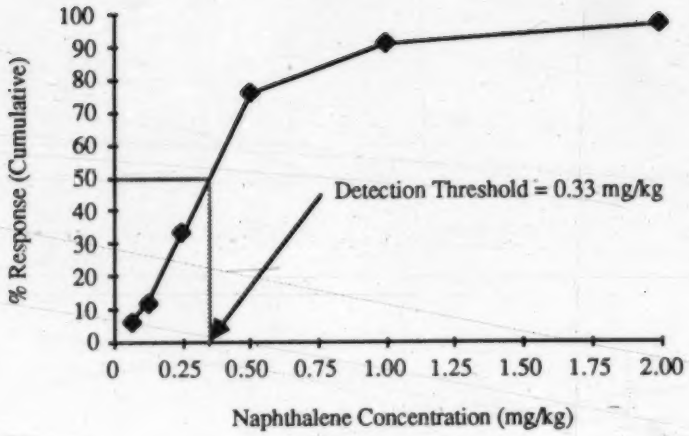


Figure 23. Detection threshold for naphthalene (n=33).

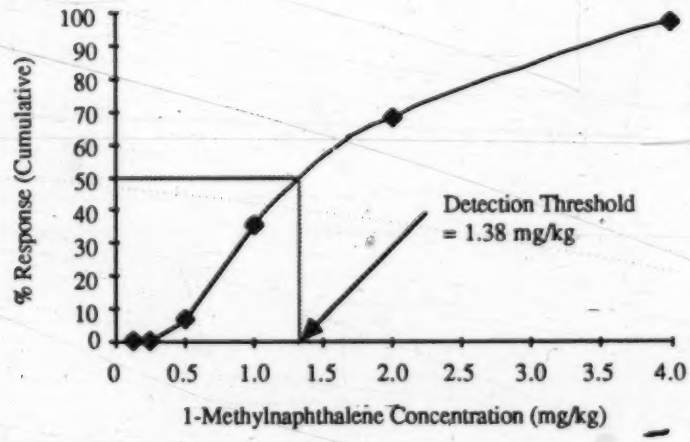


Figure 24. Detection threshold for 1-methylnaphthalene (n=31).

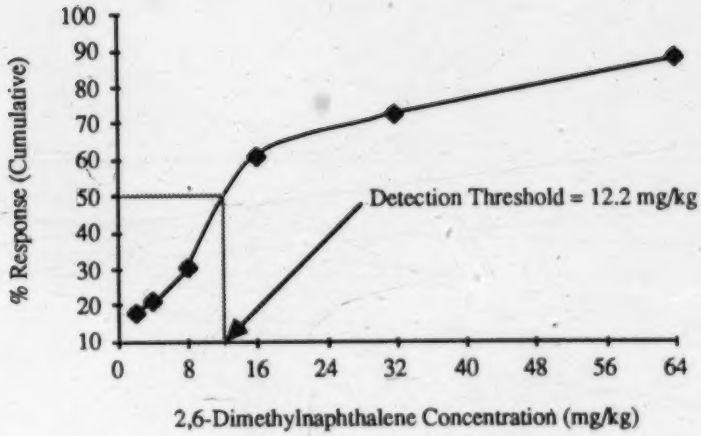


Figure 25. Detection threshold for 2,6-dimethylnaphthalene (n=33).

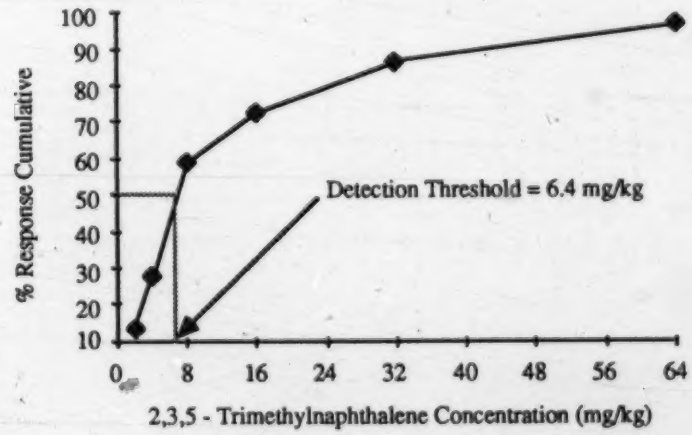


Figure 26. Detection threshold for 2,3,5-trimethylnaphthalene (n=29).

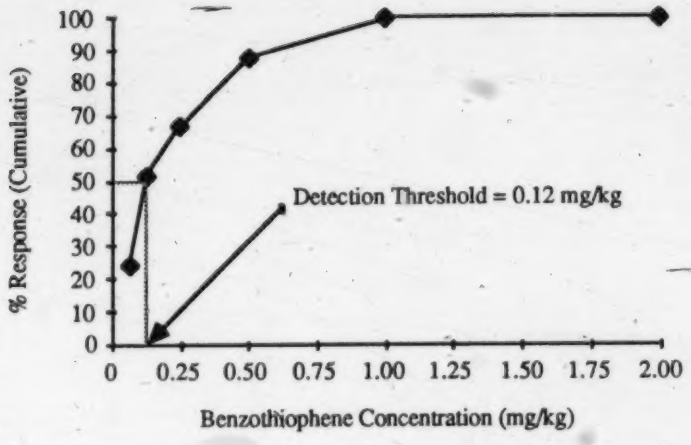


Figure 27. Detection threshold for benzothiophene (Test #1) (n=33).

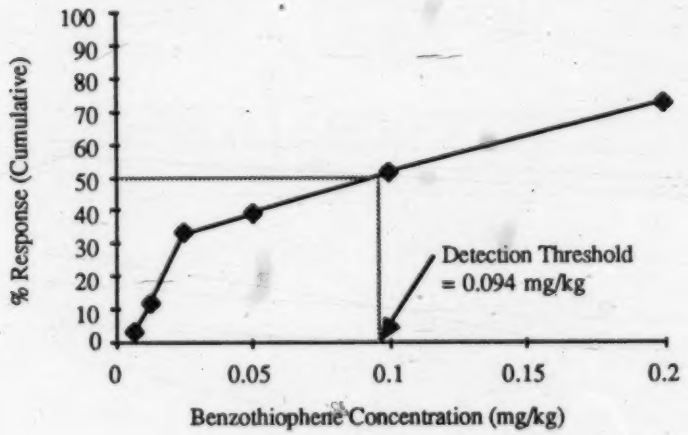


Figure 28. Detection threshold for benzothiophene (Test #2) (n=33).

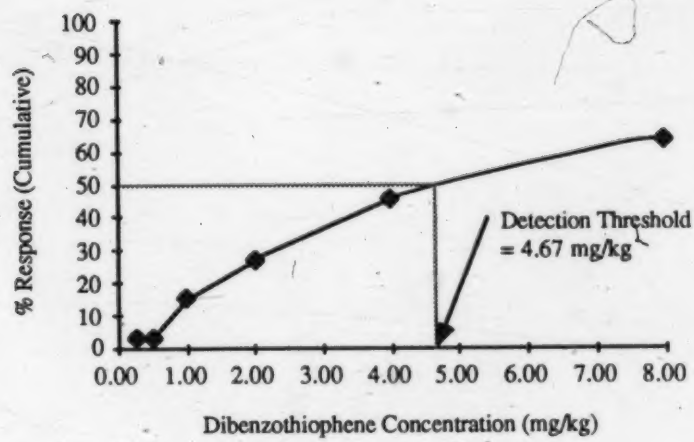


Figure 29. Detection threshold for dibenzothiophene (n=33).

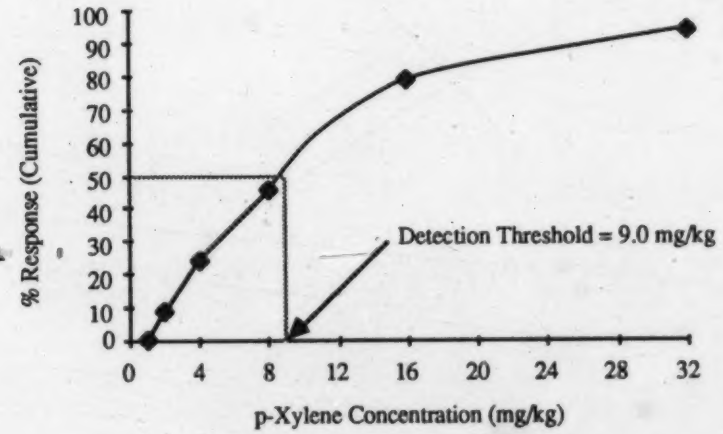


Figure 30. Detection threshold for p-xylene (n=33).

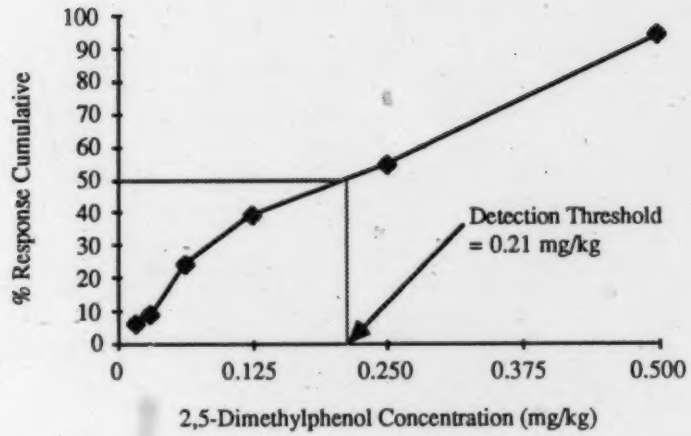


Figure 31. Detection threshold for 2,5-dimethylphenol (n=33).

sensation when no odour was detected indicated that this occurred with a few of the compounds tested (2,5-dimethylphenol was the only compound specifically mentioned). This concurs with Appel (1985), who stated that aroma, as perceived in the nasal passages, contributes 75-80% of the impression of flavour in food. Further support is provided by Kerhoff (1974, cited in Connell and Miller 1981), who found that petroleum hydrocarbons have little capacity to generate a taste sensation, and that the prime response is olfactory. Therefore, the threshold values determined in this study would be most accurately designated as odour thresholds only.

In the threshold evaluations for all compounds, some panelists either detected the compound at the lowest concentration, or could not perceive it at the highest concentration. Therefore, as in the comparison of sensory methods, the detection thresholds cannot be reported as a concentration range.

Two separate threshold evaluations were done on benzothiophene to confirm test results and compare carrier solvent methodologies. Test #2 used mineral oil rather than ethanol as a carrier solvent, and was done at a range of concentrations one order of magnitude lower than Test #1. The small difference in the observed detection levels is well within the discrimination limits of the test procedure. This suggests that the determined threshold values are independent of the range of concentrations used, and that the nature of the carrier solvent was not affecting the sensory evaluations at low concentrations.

4.3.1 Comparison with Compound Volatility

The physical properties affecting the volatility of the compounds, and hence the flavour aroma generated by each, are discussed in Section 2.4.2 and

summarized in Table 5. A comparison of these values with the odour threshold values for the compounds allows for some general observations on the correlations between the values. Threshold values appear to be unrelated to water solubility. This is probably because the compounds were spiked in fish tissue and the relative water concentration was low. As the Henry's Law Constants are derived from solubilities, it is not surprising that there is also no evident correlation between these and the threshold values.

Better correlation appears to exist between the threshold values and the vapour pressures and molecular weights of the compounds. Vapour pressure indicates the tendency of the compound to evaporate. Compounds with an extremely high vapour pressure, such as thiophene, 2-methylthiophene, toluene, p-xylene and mesitylene evaporate very quickly on being exposed to air. Their odour would dissipate very quickly, possibly explaining why these compounds could not be readily detected by the panelists. At the other extreme, compounds with a relatively low vapour pressure, such as 2,6-dimethylnaphthalene, 2,3,5-trimethylnaphthalene and dibenzothiophene, would tend to remain in the tissue much longer. Hence, these compounds tend to have a high detection value. Compounds intermediate between these two extremes, such as naphthalene, benzothiophene and 2,5-dimethylphenol, tend to be detected at very low levels. Although these general observations appear to partially explain the observed threshold values, Jones (1955, cited in Amerine et al. 1965) suggested that a perfect relationship between vapour pressure and odour threshold cannot be expected in all cases, because of steric hindrance (hindrance of chemical action ascribed to the arrangement of atoms in space) and specific structural properties of compounds which may influence adsorption on the olfactory receptors.

In general, the characteristic odour of a chemical compound decreases with molecular weight in a homologous series (Amerine et al. 1965). This is evident in the increased detection threshold level with the addition of one or two methyl groups to naphthalene. However, the detection threshold for trimethylnaphthalene was less than that of dimethylnaphthalene. The difference between these thresholds is 5.8 mg/kg, which is less than a factor of two increase from the 6.4 mg/kg threshold of trimethylnaphthalene. As previously discussed, this amount of increase in concentration is generally acknowledged to be the minimum difference in intensity perceptible to the human sense of odour. Therefore, the lower value for trimethylnaphthalene may not be significantly different from the value for dimethylnaphthalene.

Benzothiophene showed the lowest detection threshold level of the compounds tested. It is a volatile and pungent compound, with a distinctive odour. A considerably higher threshold detection value was noted for the larger and less volatile dibenzothiophene.

A high detection threshold value was noted for p-xylene. However, the oxidized form of p-xylene, 2,5-dimethylphenol, exhibited a low detection value, suggesting that the dimethylphenols may be a more important tainting component than the xylenes. Phenols may occur in the muscle tissue as a result of direct uptake, or by metabolic oxidation of the alkylated benzenes. Aquatic organisms have been shown to possess oxidative metabolic pathways for aromatic compounds (Sæthre et al. 1984) and it is possible that p-xylene is being metabolized by the fish to 2,5-dimethylphenol. Metabolic oxidations of this nature have been reported for the transformation of naphthalenes and methylated naphthalenes to the corresponding naphthols in several fish species (Melancon and Lech 1979; Varanasi

and Gmur 1981) Thomas and Rice (1982) found metabolites of alkylated benzenes in the muscle of exposed fish, suggesting that the metabolism of these compounds may occur in many tissues other than the liver.

4.3.2 Comparison with Published Threshold Values

In general, the odour threshold values determined for these compounds in fish tissue exceeded the recent reported odour threshold values in water (Table 3) by at least two orders of magnitude. Earlier reported values (pre-1970) were excluded from comparison because of the great advances in analytical detection limits made since their determination.

Again, the outdated nature of the references makes it difficult to compare the threshold values determined in this research with those values published for threshold odour concentration in fish (expressed as exposure concentration in water) (Table 2). The only recent value is reported for xylene (unknown isomer) in unknown fish tissue as 0.3 mg/L. If multiplied by the estimated bioconcentration factor for p-xylene of 146 (Table 6), the resulting tainting concentration would be 44 mg/kg in fish flesh. This value is approximately five times higher than that determined in these investigations.

The only reported threshold value for these compounds in fish tissue was for xylene (unknown isomer) in scallops at 100 mg/kg (Table 1). This is approximately eleven times higher that the value determined in this study.

4.3.3 Comparison with Bioconcentration Factors

In general, the observed threshold values are positively correlated with the estimated bioconcentration factors (Table 6). With the exception of toluene, p-

xylene and thiophene, threshold values tend to increase as bioconcentration potential increases. This suggests that, for incidents involving low level chronic discharge, compounds such as naphthalene, benzothiophene and dimethylphenol may cause short-term tainting problems because of their low detection threshold values, while compounds such as the di- and trimethylnaphthalenes, and dibenzothiophene may have long-term tainting effects because of their accumulation to higher concentrations in the fish flesh. However, in large-scale spill incidents, the first group of compounds may be primarily responsible for causing taint in fish.

This contention has been supported by Neff (1987). He claims that low molecular weight aromatics, such as benzene and naphthalene, are accumulated and released rapidly by aquatic organisms, whereas higher molecular weight compounds are accumulated more slowly, but are retained for a longer time. As a result, organisms exposed to oil tend to accumulate higher concentrations of high molecular weight hydrocarbons than those of low molecular weight hydrocarbons, even though the oil may contain higher concentrations of the latter.

4.4 SENSORY PANEL PERFORMANCE

4.4.1 Comparison of Mean Threshold Values Between Panelists

A Randomized Complete Block Design (RCBD) two-way Analysis of Variance (ANOVA) was used to test the null hypothesis of no difference in mean threshold values between panelists. Each replication of the threshold evaluation was considered a "block", and the nature of the samples and the test conditions within the block were expected to be relatively homogeneous. This was felt to be a reasonable assumption, as the samples for each replication were prepared at one

time, and all evaluations were performed at approximately the same time of the day. Each panelist was considered a "treatment". This design has the advantage of eliminating any variation between replications from comparison of the panelist evaluations.

The ANOVA was performed for every compound evaluated for threshold detection levels. The experimental F values obtained for every analysis are summarized in Table 20. Full ANOVA tables are given in Appendix C. As in the t-test evaluations in Section 4.1.3, threshold values that were above the concentration range presented were assumed to be at the next concentration interval.

Significant F values were obtained for naphthalene, 2,6-dimethylnaphthalene and benzothiophene (Test #2), indicating significant differences in panelist threshold values for these compounds.

4.4.2 Variability in Detection Threshold Values

Intra-individual changes in the threshold sensitivity of panel members during the three sessions were compared for two compounds by assessing the number of times the same detection threshold level was reported by each panelist. This procedure was used by Zoeteman (1980) to evaluate differences in odour sensitivity in threshold detection of individual compounds in water. The percentage of panel members reporting the same threshold in all three sessions, the same threshold in two sessions, and different thresholds in all three sessions were compared. Table 21 summarizes the results obtained using this procedure for the nine compounds evaluated for this investigation.

The overall consistency of evaluations was poor, with only 8.1% of the panelists reporting the same threshold for all three replications of the test. The

Table 20. Calculated F values for each compound using a Randomized Complete Block Design ANOVA. The test replications were considered as "blocks" and the panelists as "treatments".

Compound	$F_{10,20}$	Significant
Naphthalene	3.238	Yes
1-Methylnaphthalene	1.414	No
2,6-Dimethylnaphthalene	2.576	Yes
2,3,5-Trimethylnaphthalene	1.538	No
Benzothiophene (Test #1)	0.955	No
Benzothiophene (Test #2)	2.640	Yes
Dibenzothiophene	1.083	No
p-Xylene	0.967	No
2,5-Dimethylphenol	2.195	No

$F_{.05,10,20} = 2.35$ (Kennedy and Neville 1976)

Table 21. Variation in panelist consistency of taste and odour sensitivity between compounds.

Compound	Same Threshold in 3 sessions	Same Threshold in 2 sessions	Different Threshold in all sessions
Naphthalene	0%	45.5%	54.5%
1-Methylnaphthalene	18.2%	36.3%	45.5%
2,6-Dimethylnaphthalene	9.1%	54.5%	36.4%
2,3,5-Trimethylnaphthalene	0%	54.5%	45.5%
Benzothiophene (Test #1)	18.2%	18.2%	63.6%
Benzothiophene (Test #2)	9.1%	54.5%	36.4%
Dibenzothiophene	0%	54.5%	45.5%
p-Xylene	0%	54.5%	45.5%
2,5-Dimethylphenol	18.2%	18.2%	63.6%
TOTAL	**8.1%**	**43.4%**	**48.5%**

largest percentage of panelists reported different thresholds for all three sessions, further illustrating the instability of panelist sensitivity. However, panelists seldom differed by more than one concentration interval, or a factor of two, in their evaluations. Although this was assumed in establishing the concentration ranges to be the least amount of difference in intensity that can be recognized by the panelist, the actual difference thresholds for some compounds may be more than a factor of two higher than the detection thresholds. This may account for some of the variability between threshold evaluations.

Naphthalene, benzothiophene (Test #1) and 2,5-dimethylphenol exhibit the most variation in panelist consistency, with the majority of the panelists showing different thresholds in all three replications. Panelist consistency does not appear to be related to significant differences in threshold values between panelists, as determined in Section 4.4.1.

This procedure was also used to compare the consistency of individual panelists (Table 22). The panelists were ranked from 1 to 10 based on the consistency of their evaluations. To determine overall sensitivity ratings, the sensitivities were rated for each panelist for each compound, and the final rating based on a ranking of the average sensitivities. These results show that consistency is not necessarily comparable to sensitivity, as the panelist ranked #1 for consistency also exhibited a poor detection sensitivity. Observation of the panelists indicated that many approached the evaluations as a "test" of their abilities, and were more prone to guessing. The panelists exhibiting the least variability tended to give a more conservative evaluation of differences in taste and odour.

Panelist #6 was the least consistent, having never reported the same detection threshold for all replications of every compound evaluation. This panelist

Table 22. Variation in consistency of taste and odour sensitivity between panelists.

Panelist	Same Threshold in 3 sessions	Same Threshold in 2 sessions	Different Threshold in 3 sessions	Consistency Rating	Sensitivity Rating
1	0%	44.4%	55.6%	8	10
2	0%	55.6%	44.4%	7	5
3	11.1%	22.2%	66.7%	6	3
4	11.1%	55.6%	33.3%	4	7
5	22.2%	55.6%	22.2%	1	9
6	0%	0%	100.0%	10	8
7	11.1%	44.4%	44.4%	5	5
8	0%	33.3%	66.7%	9	4
9	22.2%	44.3%	33.3%	2	2
10	11.1%	66.7%	22.2%	3	1
11	0%	55.6%	44.4%	7	6
TOTAL	**8.1%**	**43.4%**	**48.5%**		

became pregnant at the beginning of the sensory threshold determinations. In their recent national survey, Gilbert and Wysocki (1987) found that, contrary to common opinion, olfactory sensitivity may be reduced rather than heightened during pregnancy.

Although more extensive training may have increased the consistency of the panelist evaluations, this was precluded by the number of compounds evaluated in this study, and the resulting time contraints.

4.5 TISSUE ANALYSIS RESULTS

Tissue analysis was completed for the naphthalene, 1-methylnaphthalene, benzothiophene (Test #1), dibenzothiophene and 2,5-dimethylphenol samples. Interference in the gas chromatograms caused by the mineral oil carrier prohibited analysis of the 2,6-dimethylnaphthalene, 2,3,5-trimethylnaphthalene, benzothiophene (Test #2) and p-xylene samples. Although mineral oil was also present in the 2,5-dimethylphenol samples, repeating the separation and cleanup procedures was effective in eliminating this interference. This procedure was not successful for the dimethylnaphthalene, trimethylnaphthalene, benzothiophene (Test #2) and p-xylene samples. The analysis for p-xylene was further hampered by the poor extraction recovery exhibited for this compound in the quality control spikes.

The analyzed concentrations of each compound in the walleye tissue were adjusted for the extraction recoveries determined from the quality control samples. The extraction values, extraction recoveries and adjusted analytical values are summarized in Table 23 for the compounds analyzed. Where possible, the extraction recoveries are given for the exact GPC column on which the sample was run. An average of the extraction recoveries for the six columns is used where the

Table 23. Fish tissue analysis results for each spiked sample.

Samples	Measured Conc. (mg/kg)	Extraction Recovery (%)	Adjusted. Conc. (mg/kg)
Naphthalene			
0.063	0.027	82	0.033
0.125	0.064	82	0.078
0.250	0.138	82	0.169
0.250	0.118	82	0.144
0.500	0.275	82	0.335
1.000	0.386	82	0.471
2.000		82	
1-Methylnaphthalene			
0.125	0.081	81	0.100
0.250	0.150	81	0.185
0.500	0.280	81	0.346
1.000	0.549	81	0.678
2.000	1.480	81	1.827
4.000	2.993	81	3.695
Benzothiophene (Test #1)			
0.063	0.047	80	0.058
0.125	0.107	70	0.153
0.125	0.094	77	0.122
0.250	0.178	90	0.198
0.500	0.272	77	0.354
1.000	0.729	77	0.947
2.000	1.285	77	1.669
Dibenzothiophene			
0.25	0.222	81	0.274
0.50	0.348	81	0.430
1.00	0.715	81	0.883
2.00	1.384	81	1.709
4.00	2.675	81	3.302
4.00	3.618	81	4.466
8.00	6.709	81	8.283
8.00	6.894	81	8.511

continued . . .

Table 23. Concluded.

Samples	Measured Conc. (mg/kg)	Extraction Recovery (%)	Adjusted. Conc. (mg/kg)
2,5-Dimethylphenol			
0.016	0.012	72	0.016
0.031	0.010	69	0.015
0.063	0.014	78	0.018
0.063	0.013	72	0.018
0.125	0.013	58	0.022
0.250	0.068	72	0.095
0.500	0.105	78	0.135

exact column was not known. Figures 32 through 34 compare the spiked and adjusted analytical values, showing the percent recoveries of each compound.

As the analytical values are all adjusted for the actual recoveries during the extraction and cleanup process, the calculated percent recoveries are assumed to reflect losses occurring during the spiking and blending processes.

The recovery efficiencies for the methylated naphthalenes are directly related to the relative volatilities of the compounds. Volatility generally decreases with addition of methyl groups. As expected, the most volatile compounds have the poorest recoveries because of the unavoidable loss of the compounds during the spiking and blending processes. Thus, naphthalene has the poorest recovery, and 2,3,5-trimethylnaphthalene has the best recovery of the alkylated naphthalenes investigated.

Dibenzothiophene is the least volatile of the compounds tested, and thus has the best recovery. The excellent recovery for this compound also confirms the homogeneity of the spiking technique used in the sample preparation. Benzothiophene, as a more volatile compound, has a slightly smaller percent recovery. Although benzothiophene has a higher vapour pressure than 1-methylnaphthalene, and is therefore more volatile, it has a better extraction recovery because of its greater affinity for the extraction solvents.

The poorest recovery is exhibited by the 2,5-dimethylphenol. The second level of clean-up required for this compound (which was not conducted for the quality control samples used to determine optimum extraction recoveries) is thought to be responsible for the low recovery. This compound is also the most difficult and variable to extract of the compounds analyzed.

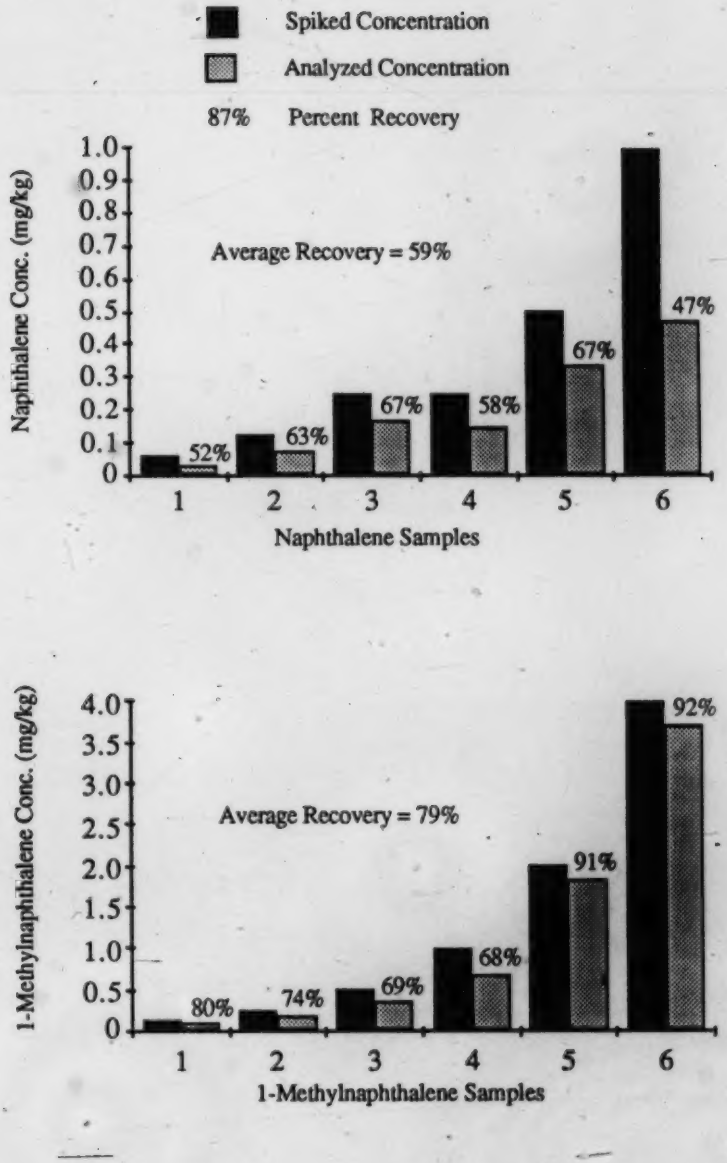


Figure 32. Comparison of spiked and analyzed concentrations for naphthalene and 1-methylnaphthalene sensory samples.

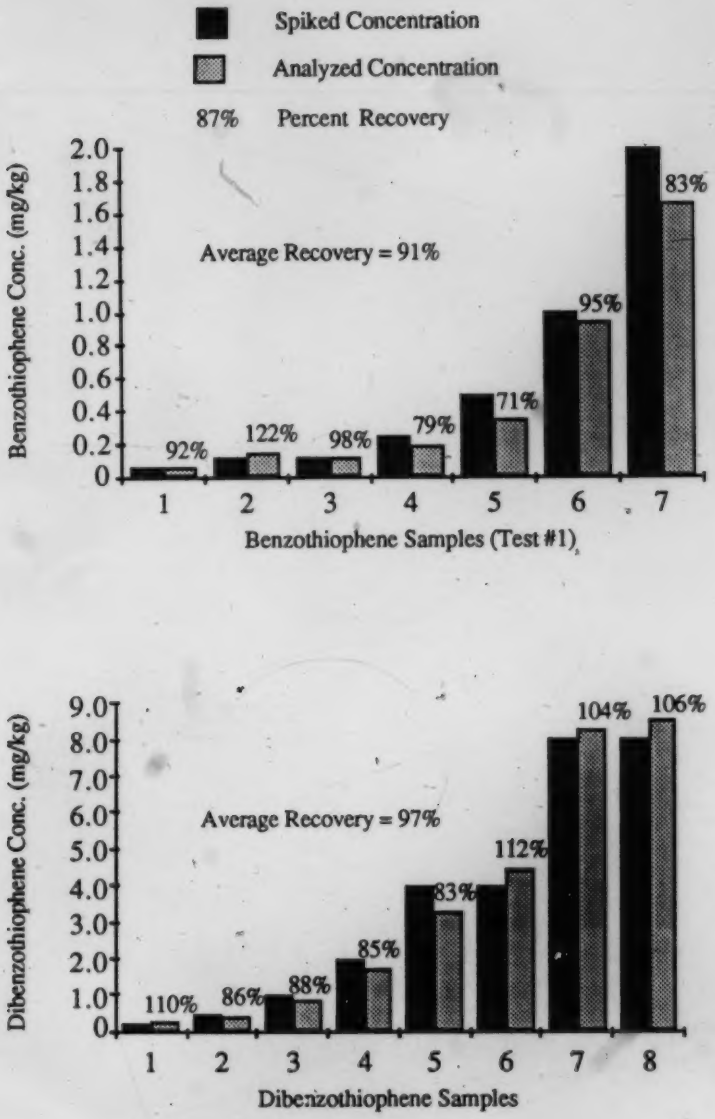


Figure 33. Comparison of spiked and analyzed concentrations for benzothiophene (Test #1) and dibenzothiophene sensory samples.

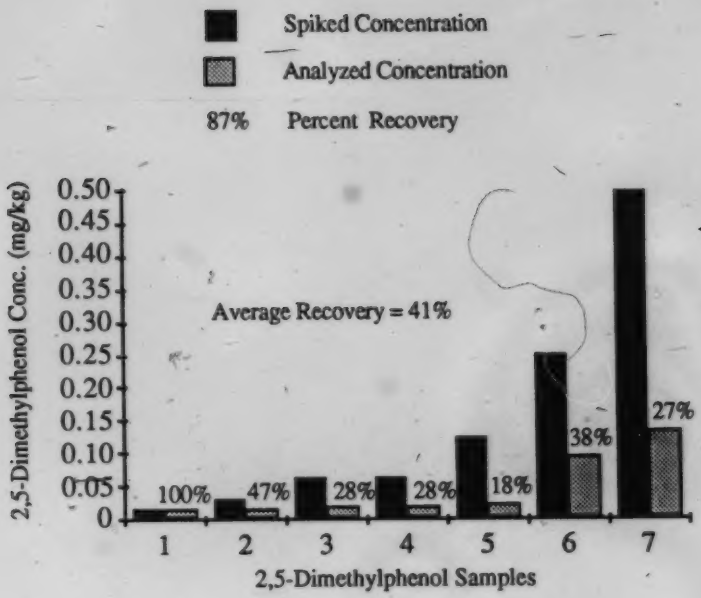


Figure 34. Comparison of spiked and analyzed concentrations for 2,5-dimethylphenol sensory samples.

Although every precaution was taken during the spiking and packaging of the samples, it is possible that some of the losses shown for the more volatile compounds may have occurred at this stage, and not just at the blending stage of the analysis. Because the panelists may have been exposed to lower concentrations than intended, the actual threshold values reported for these compounds, particularly naphthalene and 2,5-dimethylphenol, may be lower than the values reported here.

5. SUMMARY AND CONCLUSIONS

1. The threshold detection values for benzothiophene in whitefish determined in the preliminary tests done to compare sensory threshold evaluation methods were 0.335 and 0.800 mg/kg for the Consistent Series Test and the Modified Triangle Test, respectively. These values are significantly different at the 0.05 significance level using the paired t-test. The performance of the panelists was generally better for the Consistent Series Test. The Consistent Series Test was thus determined to be the more sensitive sensory threshold evaluation test. It was also the preferred method of threshold determination for the investigator and panelists because of the fewer evaluations required.

2. Comparison of detection thresholds at Replication #4 only using the paired t-test indicated no significant difference in threshold concentrations between the two methods. Differences between the testing methods may therefore be eliminated through further replications, or with increased training of the panelists.

3. The proportion of correct responses in the Modified Triangle Test that were accurately identified as being either "tainted" or "untainted" fish increased steadily from 38% in Replication #1 to 94% in Replication #4. This further reinforced the need for adequate screening and training of panelists in subsequent testing.

4. On the basis of the preliminary threshold evaluation tests, subsequent threshold evaluations were improved with respect to the preparation of the samples, test procedures, panelist screening and training, and a reduced number of samples. These improvements resulted in a lower detection threshold value for benzothiophene (0.12 mg/kg) than was determined by the Consistent Series Method in the preliminary tests (0.335 mg/kg).

5. Panelists generally scored high on the taste recognition screening tests. Salty and sweet tastes were readily recognized, while more difficulty was encountered with the sour and bitter tastes, possibly due to confusion in the terms. No panelists were ageusic.

6. Odour recognition scores showed more variability than the taste scores, probably because of the more subjective nature of the test. Panelist #7 scored less than 50% of the maximum score, and may qualify as marginally anosmic for the odours tested. The most readily recognized odour was cloves.

7. In the Sequential Analysis screening test, the cumulative score of most panelists fell within the acceptance region, as defined by the assigned specifications. The performance of all panelists exceeded the specified rejection criteria. The pairs of samples with the largest range in concentration were most frequently judged correctly.

8. The panelists selected on the basis of the three screening tests exhibited the highest consistency and sensitivity of those panelists available for the subsequent tests.

9. Thiophene, 2-methylthiophene, toluene and mesitylene exhibited relatively poor detectability by the panelists in the preliminary range determination tests. Consequently, these compounds probably do not contribute to tainting problems at low concentrations, and were not pursued for threshold detection evaluation. The detection threshold values obtained for the remaining compounds are summarized in Figures 23 through 31. Taint detection thresholds ranged from 0.09 mg/kg for benzothiophene to 12.2 mg/kg for 2,6-dimethylnaphthalene.

10. To confirm test results and compare carrier solvents, the threshold evaluations for benzothiophene were repeated at a range of concentrations one order of magnitude lower, and using mineral oil rather than ethanol as a carrier solvent for the spiking compound. The two determined thresholds were 0.12 and 0.09 mg/kg, suggesting that the determined threshold values are independent of both the range of concentrations presented and nature of the carrier solvent.

11. Detection thresholds appear to be related to the vapour pressures and molecular weights of the compounds. Other factors relating to volatility, such as solubility and Henry's Law Constant, do not appear to affect the organoleptic sensation created by the compounds in fish tissue. While those

compounds with a very low vapour pressure, such as 2,6 dimethylnaphthalene, 2,3,5-trimethylnaphthalene and dibenzothiophene, may not be readily detected because they are slow to evaporate, those compounds with a high vapour pressure, such as thiophene, 1-methylthiophene, toluene, p-xylene and mesitylene, may be equally difficult to detect because they dissipate very rapidly. Therefore, those compounds with intermediate vapour pressures, such as naphthalene, benzothiophene and 2,5-dimethylphenol, are detected at the lowest concentrations. Such compounds may also be the major causes of tainting of cooked fish arising from oil sands wastewaters.

12. A high detection threshold value was noted for p-xylene. However, the oxidized form of p-xylene, 2,5-dimethylphenol, exhibited a low detection value, suggesting that the dimethylphenols may be a more important tainting component than the xylenes. Phenols may occur in the muscle tissue as a result of direct uptake, or by metabolic oxidation of the alkylated benzenes.

13. Recent literature odour threshold values in water are at least two orders of magnitude lower than the threshold values determined in this investigation. However, recent literature threshold values in fish tissue are from 5 to 11 times higher than those determined in this study.

14. The observed threshold values are generally positively correlated with the estimated bioconcentration factors. This suggests that, although compounds such as naphthalene, benzothiophene and dimethylphenol may

be the primary cause of tainting problems in a spill situation because of their low detection threshold values, compounds such as the di- and trimethylnaphthalenes, and dibenzothiophene may have long-term tainting effects in a case of chronic low level discharge because of their accumulation to higher concentrations in the fish flesh.

15. Using a Randomized Complete Block Design two-way Analysis of Variance test, a significant difference (at the 0.05 significance level) was detected among the mean threshold values of each panelist for naphthalene, 2,6-dimethylnaphthalene and benzothiophene (Test #2).

16. In comparing the consistency of reported detection values for each compound, panelist consistency was found to be highly variable, with the majority of panelists reporting different thresholds for each replication of the test. However, the difference between individual threshold evaluations is usually within a factor of two, which is commonly accepted as the minimum detectable difference for threshold determinations.

17. A comparison of consistency of evaluation between panelists showed that this trait cannot necessarily be equated with sensitivity, as the most consistent panelist also exhibited a poor detection sensitivity. The poor performance of one panelist may be caused by reduced odour sensitivity shown to result from pregnancy.

18. The variability in panelist performance emphasizes the subjective nature of sensory testing, and the need for caution in interpreting these threshold values as an absolute threshold for each individual. Although more extensive training may have increased the consistency of the panelist evaluations, this was precluded by the number of compounds evaluated in this study, and the resulting time constraints.

19. The analyzed concentrations for those compounds that could be completed were adjusted for the known extraction recoveries of the analytical procedure. These adjusted values were then compared as a percent recovery of the original spiked concentration (Figures 32 to 34). The excellent recovery of dibenzothiophene confirms the uniformity of the spiking technique used in the sample preparation.

20. Poor analytical recoveries of a compound can be directly related to increases in volatility. Losses were probably accrued during the handling of the samples. Despite rigorous precautions taken in the spiking and packaging of the samples, some loss of the compounds may have occurred at this stage, and not just at the blending stage of the analysis. Because the panelists may have been exposed to lower concentrations than intended, the actual threshold values reported for some volatile compounds, particularly naphthalene and 2,5-dimethylphenol, may therefore be lower than the values reported here.

21. The disparity between the known, spiked concentration and the analyzed concentration for many of the compounds illustrates the need to apply caution in the interpretation of fish tissue analyses, as the analytical values will often underestimate the concentration of a specific compound in the tissue. This is particularly true for highly volatile and polar compounds.

6. RECOMMENDATIONS

Although this study produced some definitive answers on the compounds in oil sands wastewaters with the potential for fish tainting, more information is required to set treatment standards for these effluents. Suggested areas for further research are:

1. Similar determination of detection threshold values in fish flesh should be done for other compounds in oil sands wastewaters suspected of causing fish tainting to determine the classes of compounds with the greatest potential for causing taint.
2. Exposure experiments should be conducted with the compounds evaluated in this study to determine the relationship between exposure concentration and the resulting tissue concentration.
3. The interaction of combinations of compounds should be investigated to determine if any additive, synergistic or antagonistic effects on flavour impairment result.
4. Further detailed analysis of fish tissue and bile is required from fish exposed to process waters to determine the actual uptake and bioaccumulation of the compounds identified in this research
5. Consideration should be given to a representative compound, such as benzothiophene, or a representative analysis, such as HPLC bile analysis, that could be used for routine monitoring of possible fish tainting incidents.

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APPENDIX A

TASTE AND ODOUR PANELS TO INVESTIGATE FISH TAINTING

BACKGROUND

Three research programs are being conducted to investigate aspects of the fish tainting potential of hydrocarbons produced by the oil sands mining and upgrading operations in the area of the Athabasca River north of Fort McMurray. These studies constitute the M.Sc. thesis requirements for three students in the Environmental Group of the Department of Civil Engineering, University of Alberta. Ultimately, the results of these studies will assist government regulatory agencies in establishing and enforcing effluent standards for the oil sands industry.

A general objective of these programs is to correlate the subjective evaluation of taste and odours in fish flesh with objective analysis methods. The subjective component of this work requires the sensory evaluation of taste and odour levels by a trained taste panel.

Two of the studies involve the use of single, known hydrocarbon compounds. In the first study, known amounts of the compound will be used to "spike" dead fish flesh. A range of concentrations will be used to determine the threshold taste and odour levels for each panelist. In the second study, corresponding concentrations of three of these compounds will be introduced to the water used to hold live fish, and the actual uptake of these compounds will be determined. The flesh from these fish will also be evaluated by the panelists for taste and odour.

The third study will investigate the live fish uptake of dilutions of various process waters and effluents resulting from the actual oil sands extraction and upgrading operations. As the chemical components of these waters are not fully known, only odour will be evaluated by the taste panel.

TASTE AND ODOUR PANELS

The taste and odour panels are the most important component of these research projects. Humans are very sensitive measuring tools, and can detect extremely low concentrations of many chemical compounds. There is still no reliable mechanical substitute for the human senses of taste and odour. Although advanced analytical measuring devices can measure specific compounds, trained sensory evaluation panelists can supply more complex information.

The importance of the panelists to the results of these studies make it necessary to carefully screen and train panelists prior to the actual sensory evaluations. The screening process consists of two phases. The first phase measures both the ability of each panelist to distinguish the four basic tastes of sweet, sour, bitter and salty, and to distinguish and describe familiar odours. The second phase consists of a series of tests in which two actual fish samples are compared, and the panelist is asked to determine which sample is more "tainted".

Failure to "pass" the screening tests does not mean that a panelist has no sense of taste and smell, but rather that their sensory abilities are simply less sensitive and/or less consistent than the other panelists.

Panelists who successfully pass the screening tests will be required to go through training sessions to familiarize them with the testing procedure and the medium being investigated. As three studies are involved, more than one testing procedure may be used, and additional training sessions may be introduced prior to each study.

The sensory evaluation work will run from July, 1986 to December, 1986, barring any unforeseen difficulties. The sessions will normally be held on Tuesday, Wednesday and Thursday from 4:30 to 5:30 p.m. in the Sensory Testing Room, Room #2-32 in the Agriculture-Forestry Building. Under special circumstances, individual panelists could be accommodated at alternate times, although it is preferable that this practice be kept to a minimum.

Panelists who successfully meeting the screening criteria, and who attend all sensory evaluation sessions, will receive a remuneration of $300 at the completion of all taste panel work.

Panelists suffering from a head cold, or any other ailment which impairs their ability to taste and smell, may be excused from an evaluation session, but must notify one of the researchers in advance of their absence.

At any time throughout the screening or actual evaluation procedures, panelists should feel free to ask the investigators any questions they may have about the testing procedures or results. Updates on the results of the experiments will be provided periodically for the panelists' information.

The three investigators involved in this research can usually be contacted during the day at 432-3441. After hours, they may be contacted at:

Cindy Jardine	452-0291
Wendell Koning	424-5245
Edel Dromey	435-2934

PERSONAL HISTORY

Name: _____

Address: _____

_____

Telephone: _____

Age: _____ Sex: _____

Do you have any history of cardiac or respiratory ailments? (Yes or No)

If yes, please provide details below:

Do you have any allergies? (Yes or No)

If yes, please provide details below:

Were you ever a habitual smoker of cigarettes? (Yes or No)
If yes, when, approximately, did you quit?

July 22, 1986

INSTRUCTIONS TO PANELISTS

An accurate evaluation of taste and odour of a medium requires that all extraneous taste and odours be eliminated or reduced as much as possible in the testing procedure. This will become more important in the next phase of the screening process, which will involve evaluation of actual tainted fish samples. It is therefore requested that for all future sensory evaluation sessions you observe that following precautions:

1. Refrain from eating or drinking (other than water) for 30 minutes prior to each session

2. Avoid the use of perfume, aftershave or scented soaps on the day of the sensory evaluation.

Thank you.

APPENDIX B

Table B1. Summary of panelist detection thresholds for naphthalene.

Panelist No.	Detection Threshold (mg/kg)		
	Repl. #1	Repl. #2	Repl. #3
1	0.500	0.500	1.000
2	0.500	0.250	0.500
3	0.125	0.250	0.500
4	0.250	0.500	0.500
5	2.000	1.000	>2.000
6	0.250	1.000	0.500
7	0.500	≤0.063	2.000
8	0.125	≤0.063	0.500
9	0.250	0.250	0.500
10	0.500	1.000	1.000
11	0.250	0.500	>2.000

Table B2. Summary of panelist detection thresholds for 1-methylnaphthalene.

Panelist No.	Detection Threshold (mg/kg)		
	Repl. #1	Repl. #2	Repl. #3
1	0.50	2.00	4.00
2	1.00	2.00	1.00
3	2.00	4.00	1.00
4	1.00	1.00	1.00
5	4.00	4.00	4.00
6	1.00	2.00	>4.00[2]
7	0.50	4.00	2.00
8	1.00	4.00	2.00
9	2.00	2.00	>4.00
10	1.00	2.00	2.00
11	>4.00[1]	4.00	4.00

1 Not included in overall threshold evaluation - samples not warm enough

2 Not included in overall threshold evaluation - bag leaked, and samples were very wet

Table B3. Summary of panelist detection thresholds for 2,6-dimethylnaphthalene.

Panelist No.	Detection Threshold (mg/kg)		
	Repl. #1	Repl. #2	Repl. #3
1	>64.00	64.00	16.00
2	32.00	64.00	16.00
3	16.00	≤2.00	≤2.00
4	>64.00	>64.00	16.00
5	32.00	64.00	32.00
6	32.00	64.00	16.00
7	8.00	16.00	16.00
8	4.00	8.00	≤2.00
9	16.00	>64.00	8.00
10	≤2.00	≤2.00	≤2.00
11	16.00	64.00	16.00

Table B4. Summary of panelist detection thresholds for 2,3,5-trimethylnaphthalene.

Panelist No.	Detection Threshold (mg/kg)		
	Repl. #1	Repl. #2	Repl. #3
1	8.00	>64.00	64.00
2	4.00	4.00	64.00[1]
3	≤2.00	16.00	64.00[1]
4	32.00	8.00	64.00[1]
5	64.00	32.00	64.00
6	4.00	8.00	64.00[1]
7	≤2.00	≤2.00	16.00
8	32.00	16.00	≤2.00
9	8.00	8.00	16.00
10	8.00	4.00	8.00
11	32.00	8.00	8.00

[1] Not included in overall threshold evaluation - "blank" sample had noticeable off-flavour

Table B5. Summary of panelist detection thresholds for benzothiophene (Test #1).

Panelist No.	Detection Threshold (mg/kg)		
	Repl. #1	Repl. #2	Repl. #3
1	0.500	0.250	0.500
2	0.500	1.000	≤0.063
3	≤0.063	≤0.063	≤0.063
4	0.500	≤0.063	0.125
5	0.500	0.125	0.125
6	0.500	≤0.063	0.250
7	1.000	0.125	≤0.063
8	1.000	0.250	0.125
9	0.125	0.125	0.125
10	0.500	0.250	0.125
11	1.000	≤0.063	0.250

Table B6. Summary of panelist detection thresholds for benzothiophene (Test #2).

Panelist No.	Detection Threshold (mg/kg)		
	Repl. #1	Repl. #2	Repl. #3
1	0.1000	>0.2000	0.0125
2	0.2000	0.1000	0.2000
3	≤0.0063	0.0125	0.0250
4	>0.2000	0.0250	0.1000
5	0.0500	0.0500	0.2000
6	0.2000	0.0250	>0.2000
7	>0.2000	≥0.2000	0.2000
8	0.2000	>0.2000	>0.2000
9	0.0250	0.0250	0.0250
10	0.0250	0.0125	0.1000
11	0.2000	≤0.2000	>0.2000

Table B7. Summary of panelist detection thresholds for dibenzothiophene.

Panelist No.	Detection Threshold (mg/kg)		
	Repl. #1	Repl. #2	Repl. #3
1	>8.00	>8.00	4.00
2	8.00	>8.00	2.00
3	4.00	2.00	>8.00
4	8.00	8.00	>8.00
5	>8.00	>8.00	8.00
6	4.00	8.00	>8.00
7	8.00	2.00	>8.00
8	>8.00	1.00	>8.00
9	4.00	>8.00	4.00
10	4.00	1.00	1.00
11	1.00	2.00	≤0.25

Table B8. Summary of panelist detection thresholds for p-xylene.

Panelist No.	Detection Threshold (mg/kg)		
	Repl. #1	Repl. #2	Repl. #3
1	>32.00	16.00	4.00
2	16.00	16.00	32.00
3	32.00	16.00	32.00
4	32.00	8.00	32.00
5	8.00	4.00	16.00
6	16.00	>32.00	2.00
7	16.00	16.00	8.00
8	8.00	16.00	8.00
9	4.00	8.00	2.00
10	4.00	16.00	4.00
11	16.00	8.00	2.00

Table B9. Summary of panelist detection thresholds for 2,5-dimethylphenol.

Panelist No.	Detection Threshold (mg/kg)		
	Repl. #1	Repl. #2	Repl. #3
1	0.500	0.063	0.125
2	0.250	0.063	0.500
3	>0.500	≤0.016	0.500
4	0.500	>0.500	0.500
5	0.500	0.500	0.500
6	0.250	0.125	0.500
7	0.500	0.500	0.500
8	0.125	0.031	0.063
9	0.250	0.500	0.063
10	0.250	0.063	≤0.016
11	0.250	0.125	0.125

APPENDIX C

TABLES FOR RANDOMIZED COMPLETE BLOCK DESIGN TWO WAY ANALYSES OF VARIANCE

NOTE: Source "A" refers to panelists and source "B" refers to replications of the test

1. NAPHTHALENE

Two Way Anova-Block Design A: 11 Groups B: Column 12

Source	DF:	Sum Squares:	Mean Square:	F-test:
A	10	25.815	2.582	3.238
B	2	4.327	2.163	2.714
Error	20	15.943	.797	
Total	32	46.085		

A probability: $.01 < p \leq .025$ B probability: $.05 < p \leq .10$

2. 1-METHYLNAPHTHALENE

Two Way Anova-Block Design A: 11 Groups B: Column 12

Source	DF:	Sum Squares:	Mean Square:	F-test:
A	10	53.712	5.371	1.414
B	2	10.364	5.182	1.364
Error	20	75.97	3.798	
Total	32	140.045		

A probability: $.10 < p \leq .25$ B probability: $p > .25$

3. 2,6-DIMETHYLNAPHTHALENE

Two Way Anova-Block Design A: 11 Groups B: Column 12

Source	DF:	Sum Squares:	Mean Square:	F-test:
A	10	24025.636	2402.564	2.576
B	2	9853.515	4926.758	5.283
Error	20	18651.818	932.591	
Total	32	52530.97		

A probability: $.025 < p \leq .05$ B probability: $.01 < p \leq .025$

4. 2,3,5-TRIMETHYLNAPHTHALENE

Two Way Anova-Block Design A: 11 Groups B: Column 12

Source	DF:	Sum Squares:	Mean Square:	F-test:
A	10	11051.212	1105.121	1.538
B	2	2989.152	1494.576	2.08
Error	20	14367.515	718.376	
Total	32	28407.879		

A probability: $.10 < p \leq .25$ B probability: $.10 < p \leq .25$

5. BENZOTHIOPHENE (TEST #1)

Two Way Anova-Block Design A: 11 Groups B: Column 12

Source	DF:	Sum Squares:	Mean Square:	F-test:
A	10	.659	.066	.955
B	2	1.068	.534	7.745
Error	20	1.379	.069	
Total	32	3.106		

A probability: $p > .25$ B probability: $.0001 < p \leq .005$

6. BENZOTHIOPHENE (TEST #2)

Two Way Anova-Block Design A: 11 Groups B: Column 12

Source	DF:	Sum Squares:	Mean Square:	F-test:
A	10	.442	.044	2.64
B	2	.003	.002	.104
Error	20	.335	.017	
Total	32	.78		

A probability: $.025 < p \leq .05$ B probability: $p > .25$

7. DIBENOZTHIOPHENE

Two Way Anova-Block Design A: 11 Groups B: Column 12

Source	DF:	Sum Squares:	Mean Square:	F-test:
A	10	441.46	44.146	1.083
B	2	6.743	3.371	.083
Error	20	815.234	40.762	
Total	32	1263.436		

A probability: $p > .25$ B probability: $p > .25$

8. p-XYLENE

Two Way Anova-Block Design A: 11 Groups B: Column 12

Source	DF:	Sum Squares:	Mean Square:	F-test:
A	10	2404.848	240.485	.967
B	2	253.818	126.909	.51
Error	20	4975.515	248.776	
Total	32	7634.182		

A probability: $p > .25$ B probability: $p > .25$

9. 2,5-DIMETHYLPHENOL

Two Way Anova-Block Design A: 11 Groups B: Column 12

Source	DF:	Sum Squares:	Mean Square:	F-test:
A	10	1.085	.109	2.195
B	2	.094	.047	.949
Error	20	.989	.049	
Total	32	2.168		

A probability: $.05 < p \leq .10$ B probability: $p > .25$